PHLEBOTOMINE SAND FLY CONTROL: PREDICTING THE IMPACT OF ALTERNATIVE SAND FLY CONTROL METHODS, USING SIMULATION MODELLING, ON THE POPULATION DYNAMICS OF *Phlebotomus argentipes* (DIPTERA: PSYCHODIDAE) IN BIHAR, INDIA

A Thesis

by

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ABSTRACT

More cases of visceral leishmaniasis (VL) are reported in Bihar, India than any other region in the world. Attempts have been made to control the VL vector, the biting sand fly, *Phlebotomus argentipes*. Studies suggest that the insecticide fipronil, orally administered to cattle in drug-form, can control adult and larval sand flies. Hence, I developed an individual-based simulation model to estimate the effect of fipronil-treated cattle on sand fly population density in Bihar.

The model represents daily sand fly population dynamics as a function of temperature-driven processes in a Bihari village and the impact of fipronil-induced adult and larval mortality on sand fly population density. Fipronil efficacy decreases over time in response to number of days post-cattle-treatment and is dependent on host preference (cattle) and oviposition site preference (cattle feces). Simulated treatment was performed with varying percentages of cattle treated and number of annual treatment applications to predict reduction in the mean number of simulated adult sand flies by the third year of treatment.

Eight of 16 simulated treatment schemes resulted in reduction in the mean sand fly population of >50% within three years. Five of these simulations reduced the mean sand fly population by >67%, assumed to be a VL epidemic threshold. Two simulations eradicated sand fly populations. Additionally, simulated treatment schemes applied 12, 6, and 3 times per year showed an ability to suppress sand fly populations below an estimated epidemic threshold during the second and third years of treatment. Treating once per year is predicted to have little impact on vector abundance.

Simulations predicted that sand fly population density can be reduced below the estimated epidemic threshold (>67%) if fipronil treatment is applied at a minimum 3 times per year over a 3-year period, a less cumbersome approach than 12 or 6 applications per year in the field. The sand fly populations are more sensitive to uncertainties surrounding sand fly oviposition site preference than host preference, suggesting that oviposition sites be investigated and targeted for control. A fipronil field trial and extensive oviposition site survey would best validate the model's potential to predict fipronil efficacy.

DEDICATION

This thesis work is dedicated to my loving parents: Richard Michael Poché and Linda Lee Poché for encouraging me to broaden my academic horizons by seeking an advanced degree. In the absence of their undying support this would not have been possible.

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CHAPTER I

INTRODUCTION AND BACKGROUND

1.1 Literature Review

The most deadly form of leishmaniasis, visceral leishmaniasis (VL), is vector-transmitted through the bite of phlebotomine sand flies in the *Phlebotomus* and *Lutzomyia* genera. VL is a protozoan parasite, resulting in an estimated 500,000 human infections and 50,000 human fatalities annually, making it the second most prevalent parasitic killer on Earth, behind only malaria (Desjeux 2001a, b). It is considered a neglected tropical disease because it affects the most impoverished people in the world, occurring almost exclusively in developing countries. The highest global rate of occurrence is on the Indian subcontinent with approximately 67% of all human instances occurring in India, Bangladesh and Nepal in areas of extreme poverty and high population density (Chappuis et al. 2007).

Bihar is the most impoverished, most densely populated, and most VL-endemic state in India, with 90% of the Indian VL cases reported there (Singh et al. 2006). The known vector of VL on the Indian subcontinent is the sand fly species *Phlebotomus argentipes*, vector-transmitting the VL pathogen *Leishmania donovani* (Dinesh et al. 2000). VL on the Indian subcontinent is anthroponotic with humans acting as the primary reservoir host, but *P. argentipes* host preference is not exclusively human. Blood meal analysis suggests *P. argentipes* females blood feed on humans and cattle within villages, primarily (Mukhopadhyay and Chakravarty 1987, Ghosh et al. 1990, Basak et al. 1995, Palit et al. 2005, Garlapati et al. 2012). Upon taking blood meals, *P.*

argentipes mate, become gravid, and lay their eggs in organic matter, which sand fly larvae feed on. Organic matter, largely in the form of bovid dung, is prevalent in Bihari villages.

A potential vector-control measure is the oral treatment of cattle, with a systemic insecticide-based drug, such as fipronil, to control adult sand flies blood feeding on cattle and larvae feeding on cattle feces. Fipronil is a broad spectrum systemic insecticide and when applied orally to animals permeates in the blood and is excreted in the feces (Jackson et al. 2009). Laboratory studies demonstrate the potential for the systemic insecticides fipronil and imidacloprid, orally administered to rodents and livestock, to control adult and larval sand flies feeding on host blood and feces, respectively (Borchert et al. 2009, Wasserberg et al. 2011, Mascari et al. 2012, Ingenloff et al. 2013, Mascari et al. 2013, Poche et al. 2013).

Indoor residual spraying (IRS) is the current practice of control performed in Bihar, where the insecticide DDT is applied to the inner walls of homes and cattle sheds. DDT use is restricted in many developed countries and could potentially be linked to a number of human health problems including: pancreatic cancer (Cantor and Silberman 1999, Nilsen and Vatten 2000, Beard et al. 2003), prostate cancer (Dich and Wiklund 1998), colorectal cancer (Soliman et al. 1997), neurological issues (Eriksson and Talts 2000), reproductive issues (Korrick et al. 2001, Longnecker et al. 2001, Longnecker et al. 2002, Siddiqui et al. 2002, Siddiqui et al. 2003), issues regarding bone mineral density (Beard et al. 2000, Glynn et al. 2000, Derfoul et al. 2003), and immunological irregularities (Daniel et al. 2002) . Also, *P. argentipes* has been shown to develop

genetic resistance to the effects of DDT (Mukhopadhyay et al. 1990, Mukhopadhyay et al. 1996, Singh et al. 2012). Research also suggests *P. argentipes* blood feed outdoors as well as indoors and that Bihari villagers sleep outdoors during the warmer months of the year, leaving themselves susceptible to bites (Poche et al. 2011, Garlapati et al. 2012, Poché et al. 2012, Perry et al. 2013). Additionally, due to uncertainty surrounding immature sand fly habitat, sand fly control has been largely limited to insecticides which target adult sand flies. Fipronil may prove to be a safer means for controlling sand fly populations and could provide a route of targeting adult sand flies blood feeding outdoors and additionally target sand fly larvae feeding on cattle feces, neither of which are targeted by DDT IRS.

Simulation models that address sand fly control and VL transmission primarily focus on disease epidemiology in human populations. Sand flies are represented in these models in the adult stage only (Dye et al. 1992, Hasibeder et al. 1992, Dye 1996, Williams and Dye 1997). VL vector control strategies have been evaluated through use of models by simulating vector reduction to estimate the impact on the transmission rate of VL in human populations and the resulting impact on the basic reproduction number (R_o), which represents the number of secondary human infections produced by one primary human infection (Dye et al. 1992, Dye 1996, Bacaer and Guernaoui 2006, Krebs 2009, Elmojtaba et al. 2010, Stauch et al. 2011, Ribas et al. 2013, Stauch et al. 2014). This is conducted by directly reducing the vector population size to estimate the point at which the transmission rate becomes lower than the recovery rate which theoretically leads to elimination of the pathogen. This type of model represents the impact of vector

population reduction on disease epidemiology in human populations but does not explicitly represent the vector control process. More specifically, this type of model predicts that if control is applied, a certain level of vector population reduction is necessary to reduce the VL transmission rate to a point where a VL epidemic can be eliminated. It does not explicitly examine the mechanisms necessary to achieve that level of population reduction.

1.2 Objectives

The objective of my research has been to develop a model to simulate the effect of the insecticide fipronil, administered orally as a drug to cattle, on sand fly population dynamics due to increased adult and larval mortality. I first described the model which represents the temperature-dependent daily development and mortality of individuals through the egg, larval, pupal, and adult stages. I then evaluated the ability of the model to appropriately simulate sand fly population dynamics under environmental conditions typical of Bihar, India. Next, I used the model to evaluate efficacy of several fipronil-based drug treatment application scenarios representing the effect of combinations of various percentages of livestock treated and frequency of treatment application. Finally, I assessed the sensitivity of the model predictions to uncertainties regarding two parameters which will directly influence the efficacy of fipronil treatment: the percentage of adult sand fly females blood feeding on cattle and ovipositing in cattle feces.

CHAPTER II

REPRESENTING SAND FLY CONTROL, THROUGH USE OF FIPRONIL-TREATED CATTLE, USING SIMULATION MODELLING

2.1 Introduction

The Sand Fly Lifecycle

Sand flies are small true flies (Diptera: Psychodidae) with adults rarely exceeding a length of 3mm. Sand flies are holometabolous, existing in four life stages: eggs, larvae, pupae, and adults. Eggs are small, oblong-shaped, and brown, typically hatching within 5-12 days (Killick-Kendrick and Killick-Kendrick 1991). Larvae feed on organic material, going through four larval instars before pupating. The larval stage may range from two weeks to several months. Pupae initially appear a yellowish color, but gain dark coloration around the developing eyes and wings when emergence is about to take place. After emerging from pupation, adult sand flies will feed on natural sugars including tree sap and honey dew from aphids. Females additionally require a host blood meal which provides nutrition necessary to produce eggs. Sand fly females become VL-infected upon taking a blood meal from an infected host and humans become VL-infected after being bit by a VL-infected sand fly female. It is suggested that sand flies do not lay their eggs universally in the wild but rather identify indicators of habitat that is suitable for sand fly larvae (Killick-Kendrick 1999). The development times of sand flies vary depending on the temperatures to which they are exposed.

Sand Fly Temperature Dependence

Sand fly ecology is not well understood. Adult sand flies typically live no more than two weeks in the laboratory, but have been shown to live much longer under natural conditions in the field. For example, researchers in southern France re-captured a wild-caught female *P. ariasi* 29 days after it was initially captured and marked (Killick-Kendrick et al. 1984). The majority of knowledge regarding sand fly growth and development is limited to laboratory experimentation.

Many processes in insect ecology are temperature-dependent. Laboratory studies, conducted at constant temperatures, suggest that temperature drives many aspects of sand fly ecology including development, oviposition and mortality. Sand flies have the ability to survive in a variety of habitats, with leishmaniasis being present in at least 88 countries on four continents, and lower thermal limits for larvae and adults suggest sand flies are capable of surviving low winter temperatures (Theodor 1936, WHO 2015).

Studies examining the role constant daily temperatures play in development, longevity, mortality and oviposition of *P. papatasi* have suggested that the speed at which sand fly processes occur accelerates in response to temperature (Guzmán and Tesh 2000, Kasap and Alten 2005, 2006, Benkova and Volf 2007). These studies reveal a tendency for development time at all stages to decrease in response to increasing temperatures. This results in shorter generation times at higher constant temperatures, suggesting higher birth rates during the summer months. Temperature-dependence of *P. argentipes* has also been studied, although to a lesser extent (Ghosh and Bhattacharya

1989, Ghosh et al. 1992). As with *P. papatasi*, *P. argentipes* show decreases in development time of eggs, larvae, pupae and adults in response to increasing temperature. The two species share similar trends in development and survivorship (Ghosh and Bhattacharya 1989, Ghosh et al. 1992, Kasap and Alten 2005, 2006). Development time of immature sand flies (larvae in particular) is greatly affected by temperature and can last for several months at typical winter temperatures. For example, one laboratory experiment in which *P. papatasi* were exposed to constant temperatures found that the larval period lasted on average 206.74 (±13.49) days at 18 ℃ (Kasap and Alten 2005).

Sand Fly Feeding Behavior

P. argentipes is anautogenous, requiring a blood meal to produce each batch of eggs (Dinesh et al. 2008). Studies have found the species to feed almost exclusively on cattle (including domestic water buffalo) and humans. Their preference for either host has been shown to vary and they appear largely opportunistic. *P. argentipes* females are collected primarily from dwellings within villages. The blood meals of engorged wild-caught sand fly females are used as an index to determine sand fly host preference with much research being conducted in the VL-endemic Indian states of Bihar and West Bengal. Researchers conducting a study in eight districts of Bihar analyzed blood meals of 725 sand flies, collected from human dwellings and cattle sheds, by testing them against antisera of eight different animal species (Mukhopadhyay and Chakravarty 1987). The study determined that *P. argentipes* feed primarily on cattle when collected from cattle sheds (80.9%) and humans when collected from human dwellings (70.37%)

and that sand flies had a 68% probability of feeding on cattle. Researchers in eight districts in West Bengal analyzed the blood meals of 395 *P. argentipes*, collected from human dwellings and cattle sheds (Basak et al. 1995). The majority of sand flies fed on humans in human dwellings (68.8%) and cattle in cattle sheds (91.6%). Researchers conducting another study in two districts in West Bengal successfully analyzed the blood meals of 370 wild-caught blood fed *P. argentipes*. The study found that all *P. argentipes* collected from human dwellings fed on humans and the majority fed on cattle (84.3%) in cattle sheds. Researchers conducting another study in two districts in West Bengal analyzed 304 *P. argentipes* blood meals. The *P. argentipes* were collected from human homes, cattle sheds and combined dwellings, referring to dwellings cohabitated by humans and cattle (Palit et al. 2005). The majority fed on bovid blood in cattle sheds and combined dwellings. The investigators argued that *P. argentipes* is highly zoophilic, feeding heavily on cattle. Researchers conducting another study in a single district in Bihar successfully analyzed 288 *P. argentipes* blood meals using cytochrome *b* polymerase chain reaction technique (Garlapati et al. 2012). The *P. argentipes* were collected from human dwellings, cattle sheds, combined dwellings, outlying village vegetation, and poultry houses. The highest percentage of positive blood meals came from combined dwellings (31.25%), then vegetation (25.69%), cattle sheds (24.31%), human dwellings (14.93%), and poultry houses (3.82%). Approximately 50% of the *P. argentipes* took a full or partial blood meal from cattle.

Immature Sand Fly Feeding Behavior

Immature (eggs, larvae, pupae) sand fly behavior is one of the more confusing aspects of sand fly ecology due in part to the difficulty of observing them under field conditions. Laboratory reared larvae feed on organic material most often being maintained on a composted mixture of rabbit feces and rabbit pellets (Killick-Kendrick and Killick-Kendrick 1991).

Immature sand flies collected from villages in Bihar and West Bengal are typically collected in loose topsoil in and around dwellings in villages. The majority are collected from the floors of cattle sheds, suggesting that sand fly larvae may feed primarily on cattle feces (Dhiman et al. 1983, Ghosh and Bhattacharya 1991, Kundu et al. 1995, Singh et al. 2008).

Sand fly larvae are cannibalistic, often consuming each other when food is scarce (Killick-Kendrick and Killick-Kendrick 1991, Volf and Volfova 2011, Heerman et al. 2015). Additionally, they will cannibalize each other in response to increasing larval density in the presence of sufficient quantities of larval food. Cannibalism has been proposed as a means of sand fly population regulation (Srinivasan and Panicker 1992).

Fipronil as a Means of Sand Fly Control

Fipronil is a phenylpyrazole and systemic insecticide that disrupts the insect central nervous system. More explicitly, it disrupts the GABA-gated and glutamate-gated chloride channels leading to hyperexcitation of the insect nerves and muscles (Raymond-Delpech et al. 2005). Fipronil has a slow withdrawal time, with fipronil metabolites being highly lipophilic, resulting in insecticidal efficacy potentially lasting

for weeks or months (Aajoud et al. 2003, Aajoud et al. 2006). A large proportion of ingested fipronil is excreted in fecal material with approximately 18-64% of ingested fipronil being excreted in goat feces and 45-75% in rat feces in laboratory experiments (EPA 1996, WHO 1997). Additionally, it binds to soils where it has a lengthy half-life, reported to range from 128-300 days (EPA 1996, EU 2011). Fipronil displays moderate toxicity to rats with an acute oral LD_{50} estimated at 97 mg of fipronil per kg of animal body weight (EPA 1996).

Oral treatment of rodents and livestock with fipronil, at low concentrations, has resulted in significant adult and larval sand fly mortality in laboratory experiments. Fipronil-induced sand fly mortality has been observed among *P. papatasi* and *P. argentipes* feeding on treated rodents. Fipronil, orally administered to lesser-bandicoot rats (*Bandicota bengalensis*), results in quicker mortality and more long-lasting effectiveness than other insecticides of interest (ivermectin, eprinomectin, diflubenzuron) with 100% mortality of *P. argentipes* adults feeding on rodent blood and 100% mortality larvae feeding on rodent feces being observed up to 20 days-post-application with a fipronil concentration of 50 ppm (Ingenloff et al. 2013). Another study found that fipronil at a concentration of 100 ppm orally administered to hamsters controlled 100% of *P. papatasi* larvae feeding on rodent feces up to 14 days-post-application and 100% of *P. papatasi* adults feeding on rodent blood up to 28 days-post-application (Mascari et al. 2013). Fipronil is also efficacious against *P. argentipes* host-feeding on Indian cattle (*Bos indicus*) (Poche et al. 2013). At fipronil concentrations of 4.0, 2.0, 1.0, and 0.5mg per kg animal bodyweight, mortality rates of 100%, 94%, 54%,

and 23%, respectively, were observed for adults blood feeding on treated cattle 21 days-post-fipronil-application. Larvae were more greatly affected with 100% mortality being observed at all of the aforementioned fipronil concentrations for larvae feeding on cattle feces deposited 21 days-post-fipronil-application. This oral method of a fipronil-based drug application is suggested as a potential means of controlling sand flies in villages in Bihar.

Sand Fly Modelling

Mathematical modelling of sand fly control and population dynamics has largely focused on VL dynamics within human populations with sand fly adults being represented in these models. (Dye et al. 1992, Hasibeder et al. 1992, Dye 1996, Williams and Dye 1997). The focus often is to estimate the basic reproduction number of the disease, also known as the R_o value. This value represents the average number of secondary infections produced by one primary infected individual and in its simplest form is the disease transmission rate divided by the disease recovery rate. Therefore, if $R_o > 1.0$ the disease can persist. Theoretically, if $R_o < 1.0$ the disease will die out (Krebs 2009). Hence, the severity of the epidemic can be reduced by reducing transmission and/or increasing recovery. The first attempt to estimate R_o within the context of VL examined zoonotic visceral leishmaniasis (ZVL) in dogs (Hasibeder et al. 1992). This model was case specific and was applied to the ZVL situation occurring in Malta and described in further detail in later research (Dye et al. 1992). Model findings concluded that R_o estimates were difficult to obtain due to uncertainties with regard to the

proportion of VL-infected Maltese canines. They went on to make what they deemed to be a cautious R_o estimate of 11.0.

When humans act as the amplifying reservoir host for VL it is referred to as anthroponotic visceral leishmaniasis (AVL). In the presence of AVL, human reservoir hosts may can be infected with asymptomatic VL for several months or even years, not showing clinical symptoms or seeking medical attention (Singh 2002). AVL occurs almost exclusively in East Africa and on the Indian subcontinent (Desjeaux 2001b). The first mathematical model representing AVL dynamics was case-specific to Assam, India and attempted to explain the occurrence of three historical AVL epidemics occurring between 1875 and 1950 (Dye and Wolpert 1988). Specifically, the goal was to determine whether intrinsic or extrinsic factors were likely responsible for these outbreaks. It was determined that intrinsic processes such as host and vector population dynamics provided the simplest explanation for the timing of these AVL epidemics. More recently, AVL models have been developed that are specific to VL dynamics in Bihar. One model estimated the underreporting of VL cases in Bihar, estimating the R_o value for the 21 most endemic districts in the state (Mubayi et al. 2010). A later model attempted to quantify VL-dynamics based on VL-infection natural history, focusing on the dynamics of the specific pathogen responsible for AVL in Bihar, *Leishmania donovani* (Stauch et al. 2011). The model reinforced the theory that VL in Bihar is primarily driven by asymptomatic, nonclinical VL victims in human populations and suggested that treatment of clinical VL cases with antibiotics would likely have no significant impact on VL transmission. Simulation results also predicted vector control

to be more efficacious than human drug treatment or prevention strategies due to vector population reduction having more impact on the VL transmission rate. The model was later used to further estimate R_o (4.71) for the purpose of further evaluating two vector control strategies: directly killing adult sand flies through DDT indoor residual spraying and destroying sand fly breeding sites through plastering cracks and crevices in homes and cattle sheds (Stauch et al. 2014). Killing adult sand flies was represented by directly reducing the life expectancy of simulated sand flies. Breeding site destruction was represented by reducing estimates of sand fly breeding site capacity within the model. Manipulation of both of these parameters resulted in a reduction in vector population density and model results predicted that an epidemic could be eliminated with >67% reduction in vector density if sand fly life expectancy was reduced and with >79% reduction in vector density if breeding sites were destroyed.

Various models representing VL dynamics have concluded that vector control strategies have greater potential to reduce occurrences of VL than do antibiotic-treatment of patients or application of preventative measures such as bed nets (Dye 1996, Elmojtaba et al. 2010, Stauch et al. 2011, Ribas et al. 2013). However, these models do not explicitly describe the role vector control measures play in reducing sand fly numbers. Additionally, these models do not attempt to represent the immature stages of sand fly development, which will be useful in evaluating control efforts that involve larval control.

Explicit Control and Temperature Driven Sand Fly Model

The research described herein did not focus on disease epidemiology in human populations, but represented sand fly control and sand fly population dynamics in an explicit manner. This was accomplished by using a temperature-driven stage structured sand fly model to represent a village population of *P. argentipes* at all developmental stages. This allowed me to predict the potential impact of fipronil on adult sand fly population dynamics. Additionally, by using the results of a previous SIR model (Stauch et al. 2014) as a benchmark, I was able to estimate the ability of fipronil treatment to reduce sand fly populations to below an estimated epidemic threshold for VL in Bihar, India.

The objective of my research has been to develop a model to simulate the effect of the insecticide fipronil, administered orally as a drug to cattle, on sand fly population dynamics due to increased adult and larval mortality. I first described the model which represents the temperature-dependent daily development and mortality of individuals through the egg, larval, pupal, and adult stages. I then evaluated the ability of the model to appropriately simulate sand fly population dynamics under environmental conditions typical of Bihar, India. Next, I used the model to evaluate efficacy of several systemic feedthrough insecticide treatment application scenarios representing the effect of combinations of various percentages of livestock treated and frequency of treatment application. Finally, I assessed the sensitivity of the model predictions to our uncertainties with regard to the proportion of adults blood feeding on cattle and ovipositing in livestock feces.

2.2 Model Description

Overview

The model represents the lifecycle of sand flies as they develop from eggs to larvae to pupae to pre-reproductive adults to pre-oviposition adults to reproductive adults to post-reproductive adults, as well as fipronil-induced larval and adult mortality (Fig. 1). Rates of development, natural mortality, and reproduction depend on the environmental temperatures to which the sand flies are exposed. Eggs, larvae, and pupae are exposed to temperatures of the organic matter in which they develop, whereas adults are exposed to ambient temperatures. Natural mortality of larvae also depends on the density of larvae in the organic matter in which they are feeding. Fipronil-induced mortality occurs in adult flies that obtain a blood meal from fipronil-treated cattle, and in larvae that feed on feces from fipronil-treated cattle. Simulations are run on a daily time step, thus all rates and probabilities described below are calculated on a daily basis. Eggs, larvae, and pupae are represented as daily cohorts whereas adults are represented as individuals. That is, the size of each daily cohort of eggs that enter the system is monitored as these eggs develop into larvae and then into pupae. When a cohort of pupae develops to the adult stage, the resulting adults are represented as individual organisms and are followed through pre-reproductive, pre-oviposition, reproductive, and post-reproductive stages (only adult females are represented in the model).

Below I present the equations used in the model to represent the development, reproduction, natural mortality, and fipronil-induced mortality of sandflies. I describe the data analyses involved in model parameterization in the appendix.

Development

To calculate rates of development of immature stages (eggs, larvae, pupae), I drew upon results of laboratory experiments conducted under constant temperatures (Ghosh and Bhattacharya 1989, Kasap and Alten 2005, 2006) and estimated temperature-dependent development under variable temperature regimes. To estimate temperature-dependent development under variable temperature regimes, I used the following general equation: $100/n_l = K/[1 + exp(a - bx)]$ (Davidson 1944). This is a bisymmetrical, sigmoid curve with the distance between the lower and upper developmental temperature thresholds (*K*) estimated as $K = [2C_1C_2C_3 - C_2^2(C_1 + C_3)]/(C_1C_3 - C_2^2)$ where C_1, C_2, and C_3 are values for $100/n_l$ on the curve at three temperatures on the abscissa. I represented the temperature-dependent development of eggs, larvae, and pupae as:

$$C_{i,Eggs} = 0.5/\left[1 + exp\left(-0.1601 \cdot T_{i,O} + 5.6067\right)\right] \quad (1)$$

$$C_{i,Larvae} = 0.0688052/\left[1 + exp\left(-0.4754 \cdot T_{i,O} + 11.298\right)\right] \quad (2)$$

$$C_{i,Pupae} = 0.25/\left[1 + exp\left(-0.2736 \cdot T_{i,O} + 7.7067\right)\right] \quad (3)$$

where $C_{i,Eggs}$, $C_{i,Larvae}$, and $C_{i,Pupae}$ represent the contributions of the current daily temperature on day *i* toward the development of eggs, larvae, and pupae, respectively, and $T_{i,O}$ represents current temperature (℃) within the organic matter on day *i* (Fig. 2a-c).

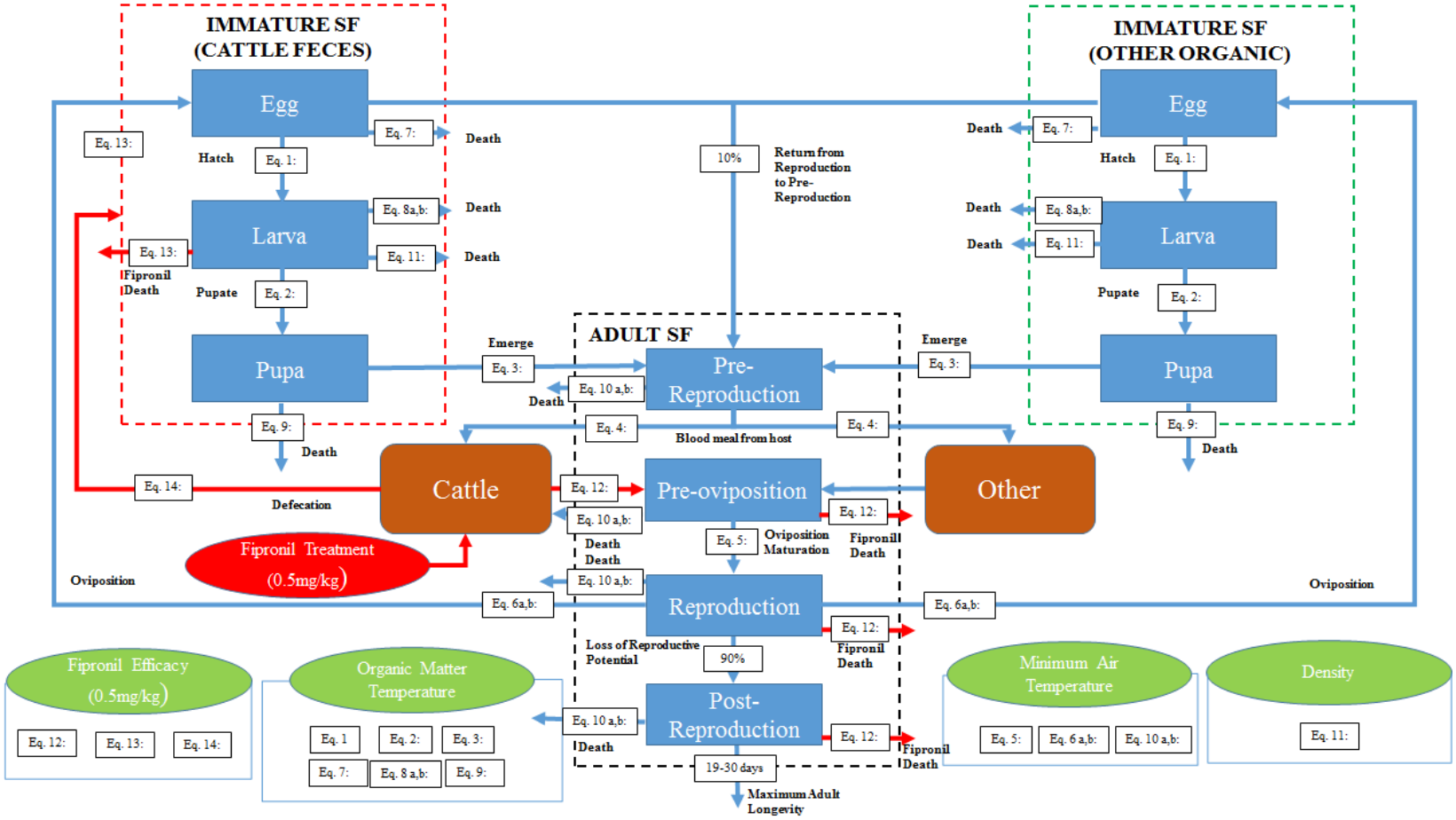


Figure 1: Conceptual model representing the impact of fipronil-induced adult and larval mortality on population dynamics of sand flies. Sand flies are represented as eggs, larvae, pupae, pre-reproductive adults, pre-oviposition adults, reproductive adults, and post-reproductive adults. Pre-reproductive adults require a blood meal to proceed with oviposition. Fipronil increases the mortality rate of adults feeding on treated cattle and larvae feeding on feces excreted by treated cattle.

The model accumulates C_i over time separately for each cohort, and when $\sum_i C_i = 1.0$ for a given cohort, the organisms in that cohort advance to the next developmental stage (Fig. 1).

After pupation, pre-reproductive adults must obtain a blood meal to advance to the pre-oviposition stage (Fig. 1) I estimated the daily probability of obtaining a blood meal based on laboratory experiments in which 0, 3, 60, 85, 94, and 96% of flies obtained their first blood meal by the end of their first, second, third, fourth, fifth, and sixth day, respectively, as an adult and used these results to develop the following curve (Srinivasan and Panicker 1993):

$$P_{i,Blood\ Meal} = 0.940321952\ /\left[1 + exp\left(-3.7061 \cdot D_{i,PE} + 10.551\right)\right] \quad (4)$$

where $P_{i,Blood\ Meal}$ is the daily probability of a pre-reproductive adult obtaining a blood meal and $D_{i,PE}$ is the number of days-post-emergence from pupation (Fig 3a).

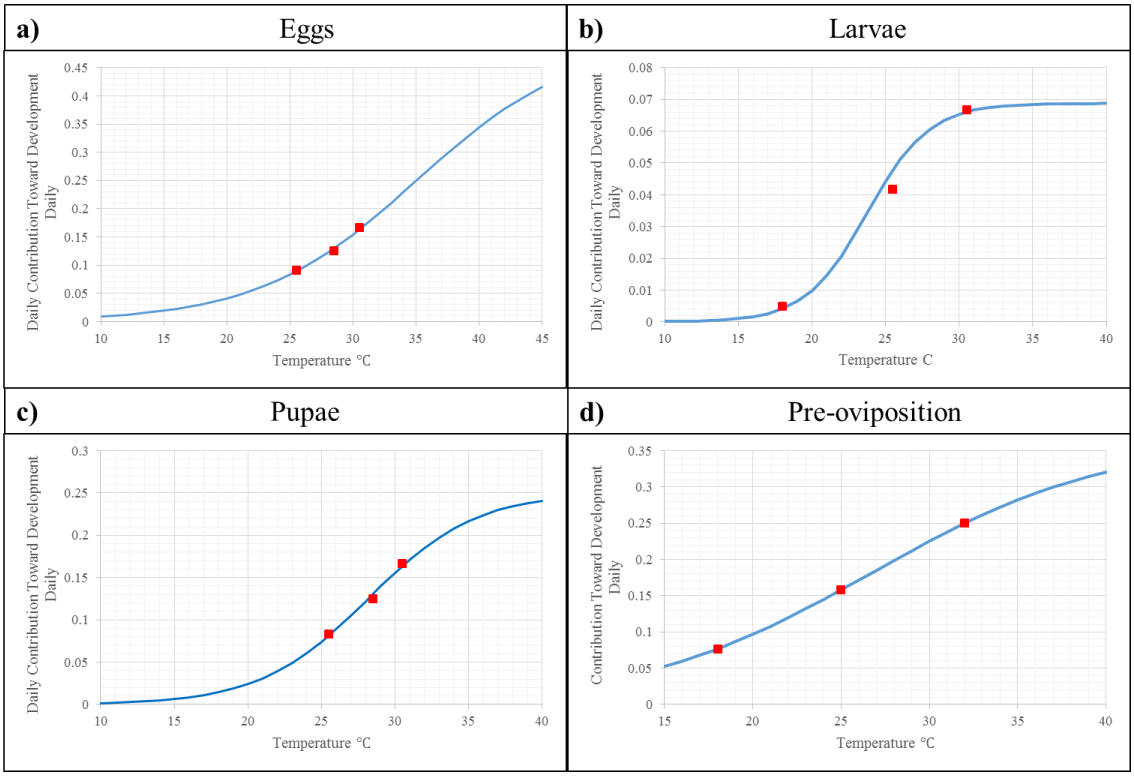


Figure 2: Curves representing of the daily contribution of temperature toward development of **a)** eggs (Equation 1), **b)** larvae (Equation 2), **c)** pupae (Equation 3), and **d)** pre-oviposition adults (Equation 5). Red squares represent the data points used to generate the curves.

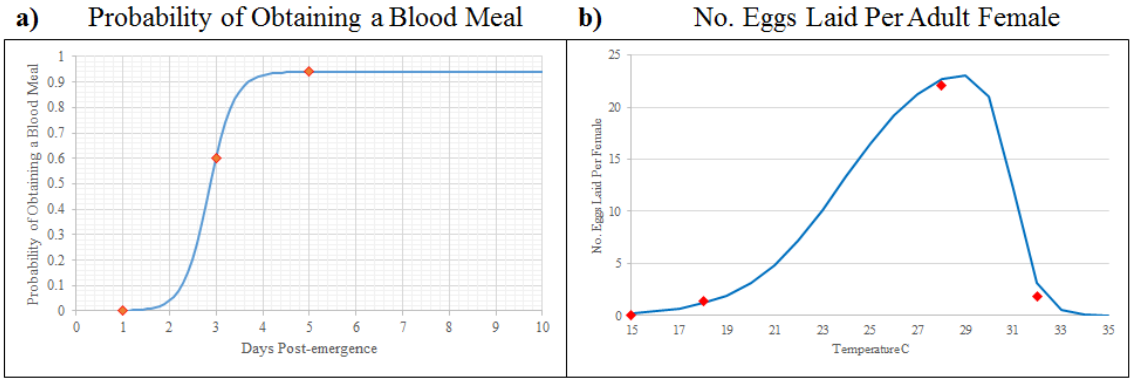


Figure 3: Curves representing **a)** the daily probability of obtaining a blood meal as a function of days-post-emergence (Equation 4) and **b)** the number of eggs laid per reproductive female as a function of current air temperature (Equation 6a,b). Red squares represent the data points used to generate the curves.

I estimated the temperature-dependent development of adults from the pre-oviposition stage to the reproductive stage in the same manner as described above for eggs, larvae, and pupae based on laboratory data (Kasap and Alten 2006):

$$C_{i,POAdults} = 0.363755/\left[1 + exp\left(-0.1503 \cdot T_{i,A} + 4.0206\right)\right] \quad (5)$$

where $C_{i,POAdults}$ is defined and calculated in the same manner as the analogous terms in Equations 1 through 3, except that $T_{i,A}$ represents current air temperature (℃) on day i rather than temperature within organic matter (Fig. 2d). When $\sum_i C_i = 1.0$, flies advance from the pre-oviposition to the reproductive stage (Fig. 1).

Reproduction

I represented the number of eggs laid per reproductive female as a function of temperature based on laboratory observations (Kasap and Alten 2006):

$$P_{i,OEaAdults} = 25.1464684/\left[1 + exp\left(-0.5238 \cdot T_{i,A} + 12.441\right)\right] \quad (6a)$$

$$P_{i,OEbAdults} = 25.1464684 - 25.1464684\ /\left[1 + exp\left(-0.5238 \cdot T_{i,A} + 12.441\right)\right] \quad (6b)$$

where $P_{i,OEaAdults}$ and $P_{i,OEbAdults}$ represent the number of eggs laid by a female at the given temperature <28.5℃ and ≥28.5℃, respectively, and $T_{i,A}$ represents the current air temperature (℃) on day *i* (Fig. 3b). Adults do not lay eggs and temperatures <15℃ (Kasap and Alten 2006).

After oviposition, reproductive females have a 90% chance of becoming post-reproductive and a 10% chance of returning to the pre-reproductive stage (Ghosh et al. 1992). If they return to the pre-reproductive stage, the daily probability of obtaining another blood meal is calculated using Equation 5, except $D_{i,PE}$ is redefined as the number of days since returning to the pre-reproductive stage.

Natural Mortality

Natural mortality of cohorts of eggs, larvae, and pupae depend on the temperature ($T_{i,O}$) of the organic matter in which they are located whereas natural mortality of adults depends on air temperatures (T_{iA}) (Fig. 4a-d). I represented the temperature-dependent natural mortality of eggs, larvae, pupae, and adults by drawing upon results of laboratory experiments (Theodor 1936, Ghosh and Bhattacharya 1989):

$$P_{i,MEggs} = 0.00052737 \cdot T_{i,O}^2 - 0.02872971 \cdot T_{i,O} + 0.39946900 \quad (7)$$

$$P_{i,MaLarvae} = 0.3898 * \exp(-0.156 * T_{i,O}) \quad (8a)$$

$$P_{i,MbLarvae} = 0.0000000000144 * exp\,(0.68195 * T_{i,O)}) \quad (8b)$$

$$P_{i,MPupae} = 0.00004973 \cdot T_{i,O}^2 - 0.00261400 \cdot T_{i,O} + 0.03635092 \quad (9)$$

$$P_{i,Ma\ Adults} = 0.5556 * \exp(-0.239 * T_{iA}) \quad (10a)$$

$$P_{i,Mb\ Adults} = 0.0005 * \exp(0.1918 * T_{iA}) \quad (10b)$$

where $P_{i,MEggs}$ and $P_{i,MPupae}$ represent the proportion of eggs and pupae dying on day *i*, $P_{i,MaLarvae}$ and $P_{i,MbLarvae}$ represent the proportion of larvae dying at temperatures <28.5℃ and ≥28.5℃, respectively, on day *i*, and $P_{i,Ma\ Adults}$ and $P_{i,Mb\ Adults}$ represent the daily probability of dying for adults at temperatures ≤10℃ and >10℃, respectively, on day *i*.

I estimated the maximum longevity of adult sand fly females in the wild to be 30 days based on field collections (Killick-Kendrick et al. 1984). If an adult fly does not suffer probabilistic temperature-dependent natural mortality after 30 days, it dies. I additionally assumed that adult flies ≥19 days old die within a day of being exposed to temperatures ≤10℃ (Theodor 1936).

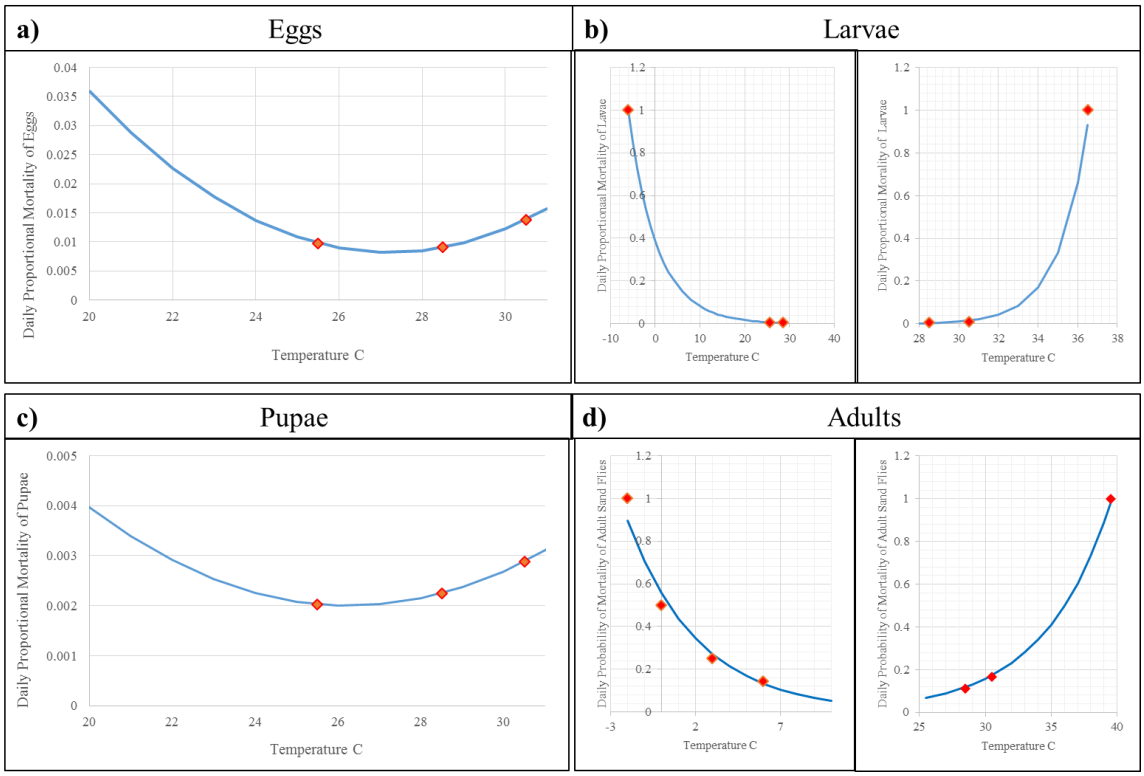


Figure 4: Curves representing **a)** the temperature-dependent natural mortality of sand fly eggs (Equation 7), **b)** larvae (Equation 8a,b), **c)** pupae (Equation 9), and **d)** adults (Equation 10a,b). Egg and pupal mortality are polynomial functional relationships whereas larval and adult mortality increases exponentially from the optimum temperatures for survivorship towards the upper and lower thermal limits. Red squares represent data points used to generate the curves.

I also represented density-dependent natural mortality of larvae based on rates of cannibalism observed in laboratory experiments conducted with different larval densities (Srinivasan and Panicker 1992):

$$P_{i,MLarvae\_C} = (1 * r_{i,Can}) * N_{i,Larvae} \quad (11)$$

where $P_{i,MLarvae\_C}$ represents the proportion of larvae dying on day *i* due to cannibalism, $N_{i,Larvae}$ represents the number of larvae in the system on day i, and $r_{i,Can}$ represents the rate of increase in the rate of cannibalism as the number of larvae increases.

Fipronil-Induced Mortality

Rates of fipronil-induced mortality depend in part on the frequency of treatment application and the proportion of the cattle treated, which I represented as management variables. Additionally, rates of fipronil-induced mortality depend on (1) the proportion of adults feeding on cattle, (2) the efficacy of fipronil contained in the blood of cattle, (3) the proportion of larvae feeding in organic matter containing cattle feces, (4) the proportion of cattle treated with fipronil, (5) the frequency of fipronil application, and (6) the efficacy of fipronil contained within cattle feces. I assumed that 50% of adult flies obtain their blood meal from cattle and that 90% of eggs are laid on, and hence larvae develop in, organic matter containing cattle feces (Singh et al. 2008, Garlapati et al. 2012).

I represented the efficacy of fipronil within the blood of cattle as decreasing exponentially as a function of the number of days after fipronil application:

$$P'_{i,MAdults} = 0.515 \cdot \exp(-0.094 \cdot D_{i,PT}) \quad (12)$$

where $P'_{i,MAdults}$ represents the daily probability of dying for an adult fly that obtained a blood meal from treated cattle $D_{i,PT}$ days post-treatment (days after application of fipronil) (Poche et al. 2013, Poche et al., unpublished data) (Fig. 5a). Once an adult

obtains a blood meal from a treated cow, I assume that its daily probability of dying due to fipronil does not change. That is, efficacy of the fipronil within the fly remains constant.

I represented the proportion of larvae dying due to fipronil within cattle feces as decreasing exponentially as a function of the number of days post-defecation (EPA 1996):

$$P'_{i,Larvae} = E_j \cdot \exp(-0.00545 \cdot D_{i,PD}) \quad (13)$$

where $P'_{i,MLarvae}$ represents the proportion of larvae dying on day i that are feeding on feces of treated cattle $D_{i,PD}$ days post-defecation ($D_{i,PD}$ days after the feces were deposited), assuming the feces were deposited j days after application of fipronil. The initial (maximum) efficacy of fipronil in cattle feces (E_j) itself decreases exponentially over time (Poche et al. 2013):

$$E_j = 0.567 \cdot \exp\left[-0.073\left(D_{i,PT} - 1\right)\right] \quad (14)$$

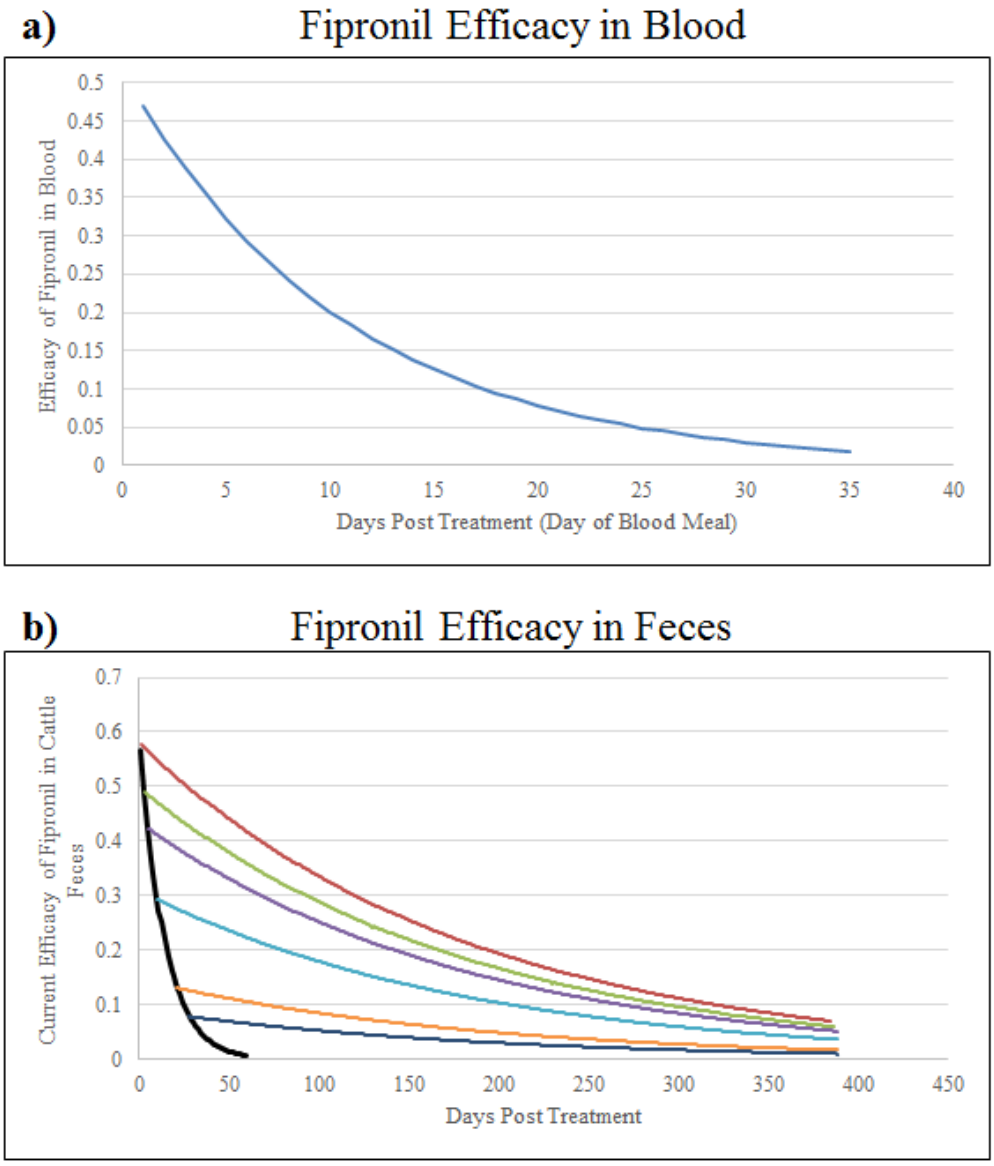


Figure 5: Curve(s) representing **a)** the decline in fipronil efficacy in blood as a function of days post-application (Equation 12) and **b)** the decline in fipronil efficacy in feces as a function of number of days post-defecation (Equation 13) (colored lines) and the number of days-post-application when defecation occurred (Equation 14) (black line).

For example, fresh feces deposited 1 day after cattle are treated have a higher efficacy than fresh feces deposited 2 days after cattle are treated (Fig. 5b). I assumed that fipronil-induced mortality and natural mortality were completely additive.

2.3 Model Evaluation

To evaluate the potential usefulness of the model in simulating the village population-level response of sand flies to fipronil-induced mortality, I first verified the model could represent adequately the rates of development, reproduction, natural mortality, and fipronil-induced mortality observed under laboratory conditions. I next calibrated the model to represent environmental conditions typical of Bihar, India such that the simulated population established a seasonally-varying, dynamic equilibrium under baseline conditions (without fipronil-induced mortality) and further calibrated the model by adjusting the parameter representing density-dependent mortality of larvae to produce a dynamic equilibrium at which the population would not naturally go extinct or increase exponentially. I then validated the baseline model by (1) evaluating the ecological interpretability of seasonal trends in the simulated sand fly life cycle and (2) comparing simulated fluctuations in abundance of adult sand flies to fluctuations observed in each of three villages in Bihar over a 12-month period. Finally, I examined the sensitivity of fluctuations in relative abundance of sand flies to changes in the time series of temperatures to which they were exposed.

Verification

The model reproduced the rates of development, reproduction, natural mortality, and fipronil-induced mortality observed under laboratory conditions reasonably well.

Development of Immatures: Eggs, Larvae, Pupae

Simulated development times of eggs, larvae, and pupae were similar to those observed in laboratory experiments (Ghosh and Bhattacharya 1989, Kasap and Alten 2005), except that simulated development times were longer at 20℃ (Fig 6a). However, the variability associated with observed development times of larvae and pupae were large. Development times simulated at temperatures encompassing the range of soil temperatures reported during a field study in West Bengal (Ghosh et al. 1999) at which I estimated immature sand flies to be exposed in the field, captured the expected non-linear increase at cooler temperatures (Guzmán and Tesh 2000, Kasap and Alten 2005), with the total development time of immature stages at 30℃ nearly 130 days longer that at 20℃ (Fig. 6b).

Development of Adults: Pre-reproduction, Pre-oviposition

Simulated lengths of the pre-reproductive stage were similar to those observed in the laboratory (Srinivasan and Panicker 1993), with the majority of simulated sand flies taking blood meals 3 and 4 days post-emergence (Fig. 7a). The simulated proportion of ovipositing females that obtained a second blood meal was similar to that of laboratory-reared females (Ghosh et al. 1992) (0.100 versus 0.093). Simulated and observed (Kasap and Alten 2006) lengths of the pre-oviposition stage were similar, with lengths decreasing in response to increasing temperatures (Fig. 7b).

Reproduction: Eggs Laid Per Reproductive Female

Simulated and observed (Kasap and Alten 2006) numbers of eggs laid per female were similar, with the by far the highest number of eggs being laid at 28℃ (Fig. 7c).

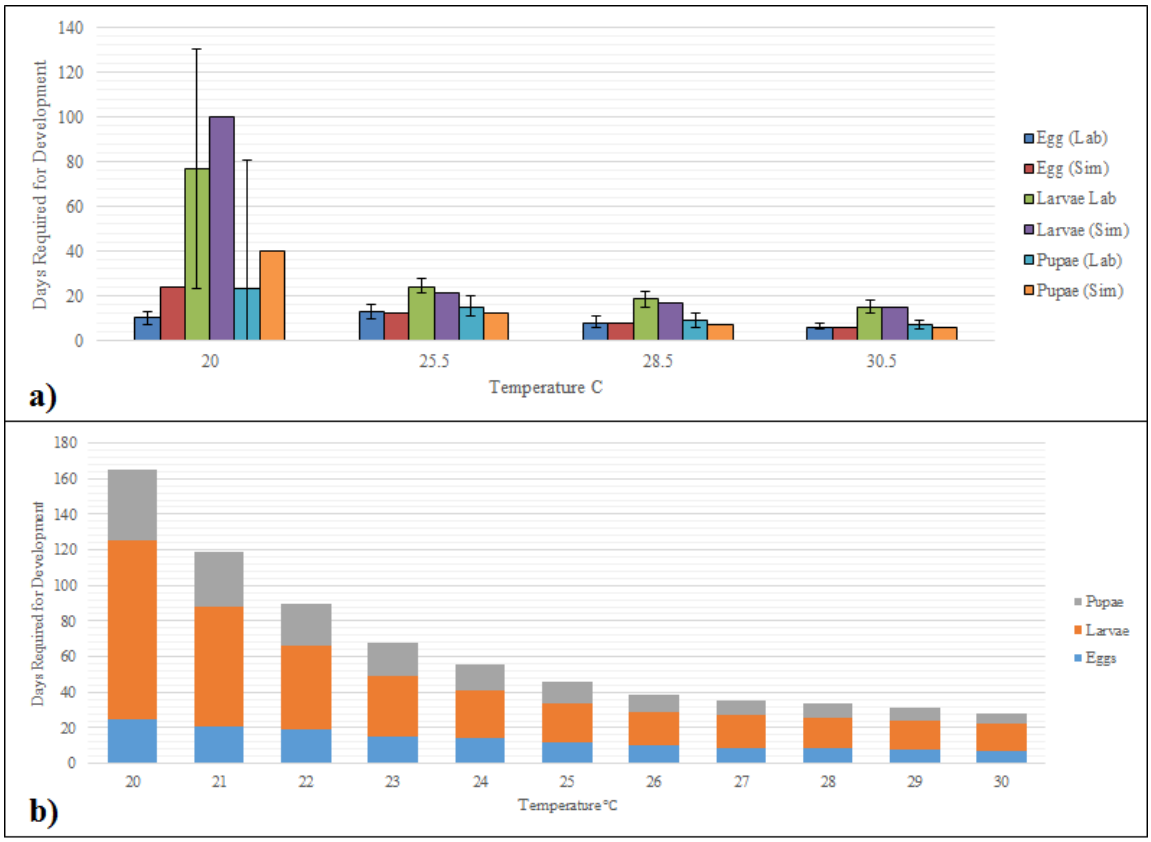


Figure 6: Comparison of **a)** simulated and observed mean development times of sand fly eggs, larvae, and pupae at the indicated temperatures, and **b)** simulated development times of eggs, larvae, and pupae at the indicated temperatures encompassing the range of soil temperatures collected during a field study in West Bengal, India (Ghosh et al. 1999) to which I estimated immature sand flies were exposed. Vertical bars in part **a** represent ±1 standard deviation of development times at 20℃ (Ghosh and Bhattacharya 1989, Kasap and Alten 2005) and the range of development times at temperatures 25.5-30.5℃.

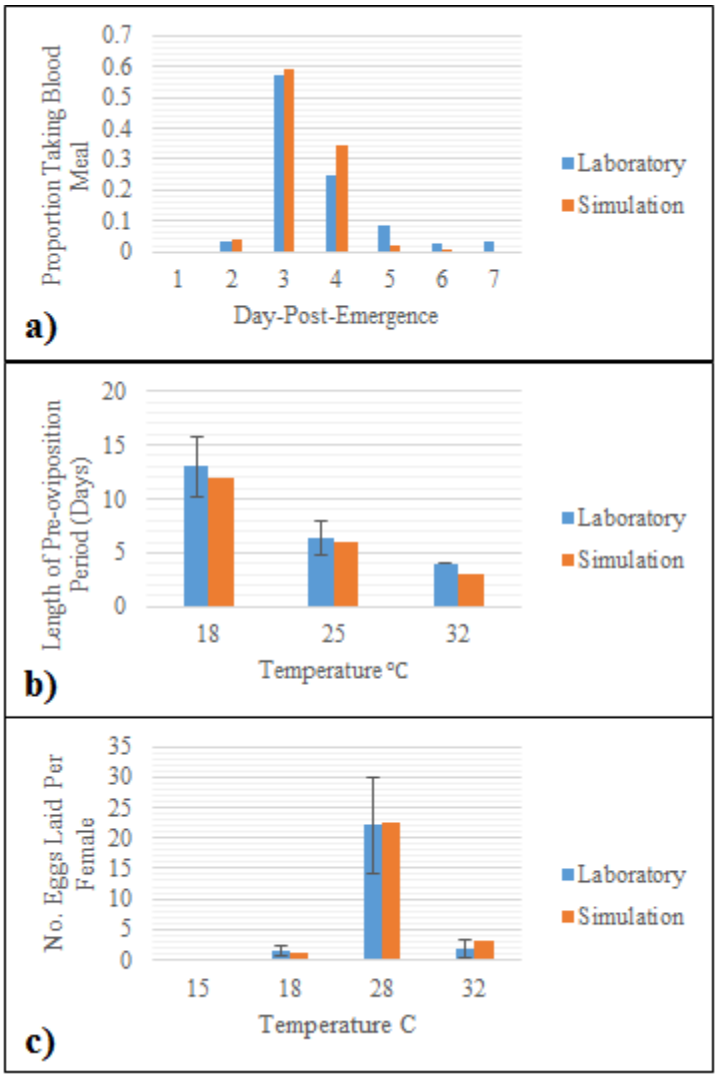


Figure 7: Comparison of **a)** simulated and observed daily proportion of blood meals obtained (Srinivasan and Panicker 1993) by pre-reproductive females as a function of days-post-emergence, **b)** simulated and observed (Kasap and Alten 2006) mean lengths of the pre-oviposition period at the indicated temperatures, and **c)** simulated and observed (Kasap and Alten 2006) number of (female) eggs laid per female at the indicated temperatures. Vertical bars represent ±1 standard deviation. The number of eggs observed in the laboratory in part **c** are divided by two in order to account only for females.

Natural Mortality

Simulated rates of natural mortality of eggs, larvae, and pupae were similar to observed natural mortality rates (Ghosh and Bhattacharya 1989), with mortality lowest at 28.5℃ and increasing at both cooler and warmer temperatures (Fig. 8a). Simulated longevities of adults were similar to longevity in the laboratory (Theodor 1936, Ghosh and Bhattacharya 1989), with longevity longest at 25.5℃ (Fig 8b).

Fipronil-induced Mortality

Simulated probabilities of fipronil-induced mortality of adults were similar to those observed (Poche et al. 2013, Poche et al., unpublished data), with the daily probability of mortality decreasing from 0.469 for adults obtaining a blood meal on the day the cow was treated to 0.072 for those obtaining a blood meal 21 days after treatment (Fig. 9a). With one exception, simulated rates of fipronil-induced mortality of larvae were similar to those observed (Poche et al. 2013), with the daily mortality rate decreasing from 0.567 to 0.132 for larvae exposed to organic matter containing cattle feces deposited from 1 to 21 days post-fipronil-application, respectively (Fig. 9b). The exception was that the 21-days post-application mortality rate in the laboratory was greater than the 14-days post-application rate, whereas the simulated mortality rate continued to decrease from 14 to 21 days post-application.

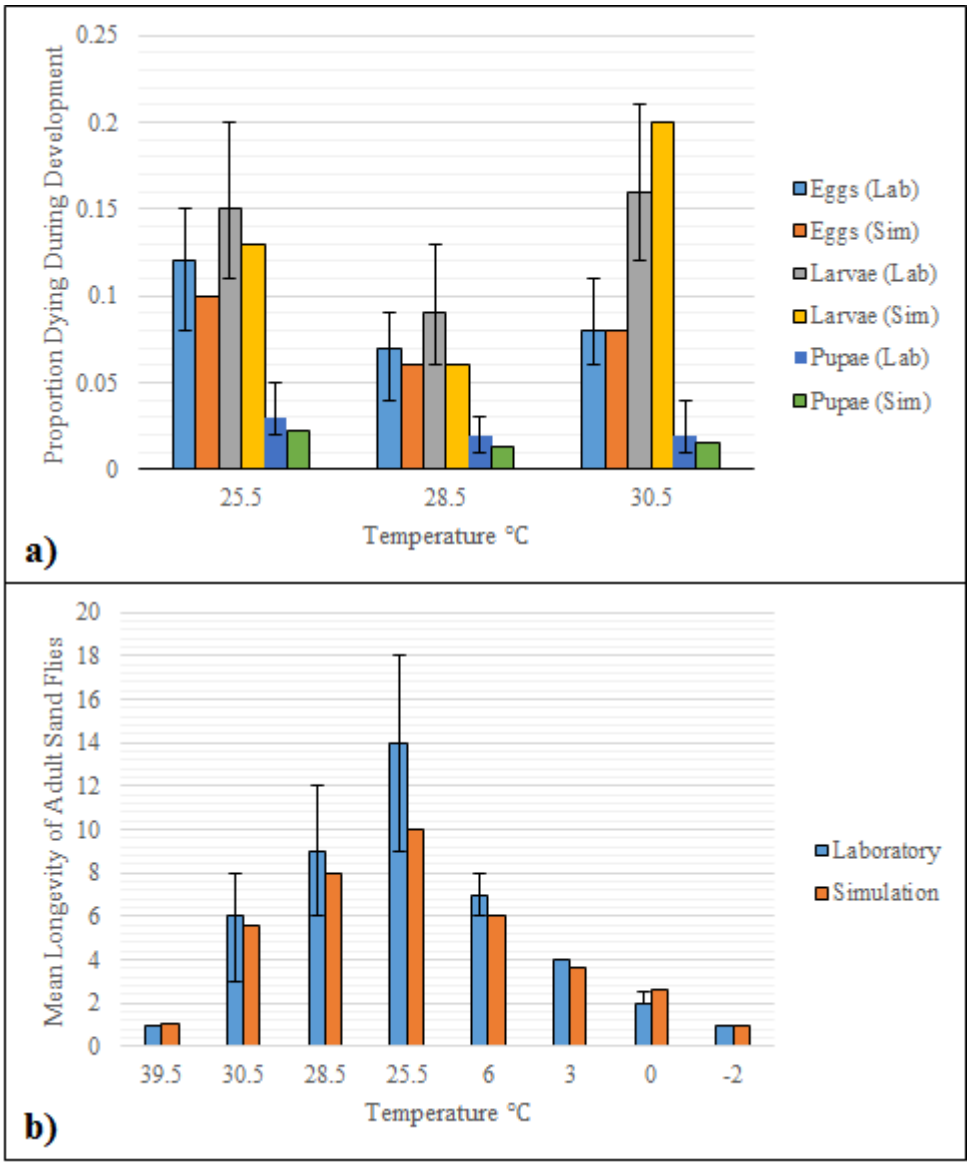


Figure 8: Comparison of **a)** simulated and observed (Ghosh and Bhattacharya 1989) natural mortality rates of eggs, larvae, and pupae at the indicated temperatures (range reported), and **b)** simulated and observed (Theodor 1936, Ghosh and Bhattacharya 1989) mean longevities of adult sand flies at the indicated temperatures. Vertical bars represent the range of values.

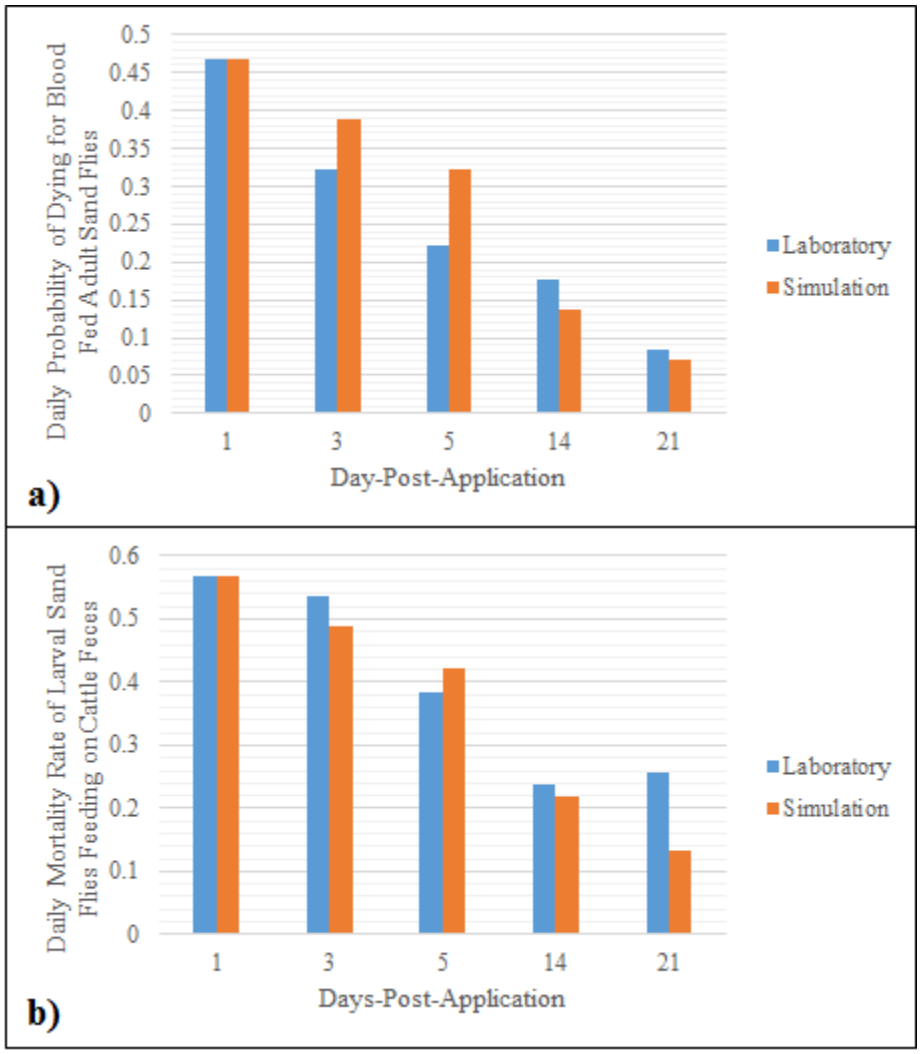


Figure 9: Comparison of **a)** simulated and observed (Poche et al. 2013, Poche et al., unpublished data) probabilities of fipronil-induced mortality of adults obtaining a blood meal the indicated number of days post-treatment, and **b)** simulated and observed (Poche et al. 2013)fipronil-induced mortality rates of larvae exposed to organic matter containing cattle feces deposited the indicated number of days post-treatment.

Calibration

I calibrated the model to represent environmental conditions typical of Bihar, India by representing annual fluctuations in (1) simulated air temperatures ($T_{i,A}$) with a time series of 365 minimum daily air temperatures recorded in a village in Bihar (Poche et al. 2011, Poche et al., unpublished data) and (2) simulated temperatures within organic matter ($T_{i,O}$) by fitting a cosine curve to a graphical representation of annual fluctuations in soil temperatures in West Bengal, India (Ghosh et al. 1999) (Fig. 10). I further calibrated the model by adjusting the parameter controlling the density-dependent mortality of larvae due to cannibalism ($r_{i,Can}$) such that the simulated population would establish a seasonally-varying, dynamic equilibrium at population levels (1) high enough to avoid chance extinctions under baseline conditions (without fipronil-induced mortality), (2) low enough to avoid excessively long simulation times and (3) high enough that the impact of fipronil-induced mortality on sand fly population dynamics would be robust to increases and decreases in equilibrium population levels. With $r_{i,Can} = 1 * 10^{-6}$ the simulated population met these three criteria, and moderate increases or decreases in this value ($1 * 10^{-7} \leq r_{i,Can} \leq 1 * 10^{-4}$) affected the equilibrium level of the adult sand fly population, but did not affect the seasonal qualitative dynamics of the model (Fig. 11a-b). With ($r_{i,Can}$) $\geq 1 * 10^{-3}$ the population could not sustain itself and with $r_{i,Can} < 1 * 10^{-8}$ the population grew exponentially. Furthermore, effects of fipronil-induced adult and larval mortality on sand fly population dynamics were robust at $r_{i,Can}$ values $\leq 1 * 10^{-5}$, indicating the resulting impact of fipronil-induced mortality

on sand fly population dynamics was not noticeably sensitive to changes in $r_{i,Can}$ when equilibrium population levels exceeded those generated by $r_{i,Can} = 1 * 10^{-4}$.

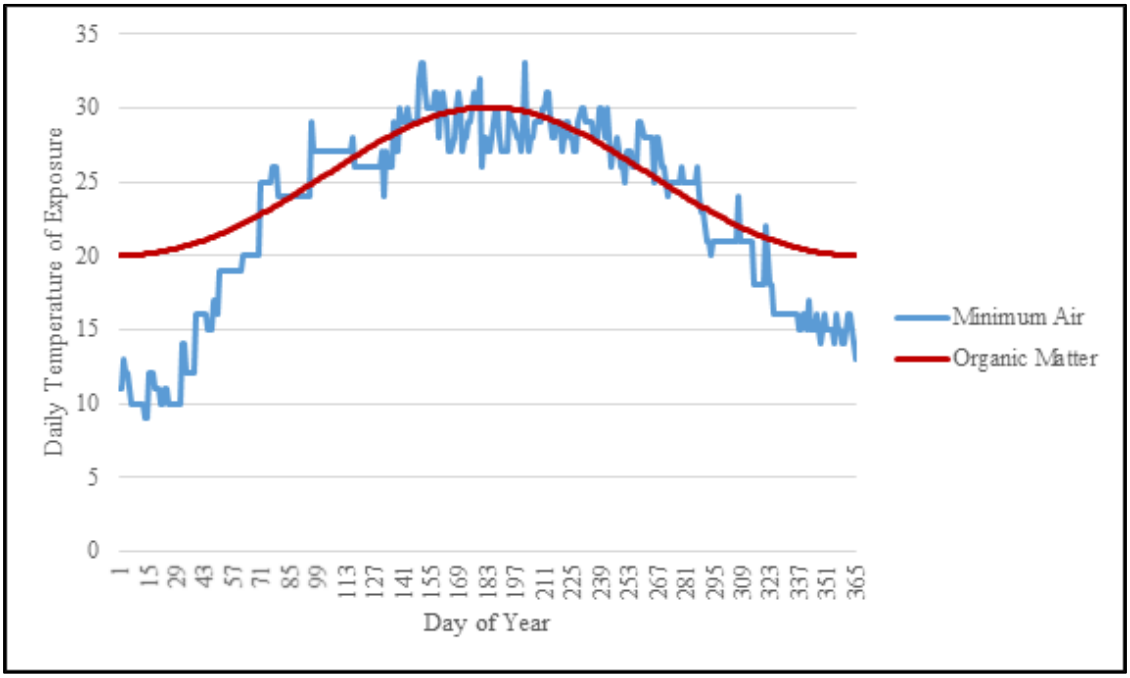


Figure 10: The daily air temperature data recorded at a village in Bihar, India (Poche et al. 2011, Poche et al., unpublished data) and a cosine curve fitted to a graphical representation of annual fluctuations in soil temperatures in West Bengal, India (Ghosh et al. 1999), which were used to calibrate the time series of air temperatures and temperatures within organic matter used in the simulation model.

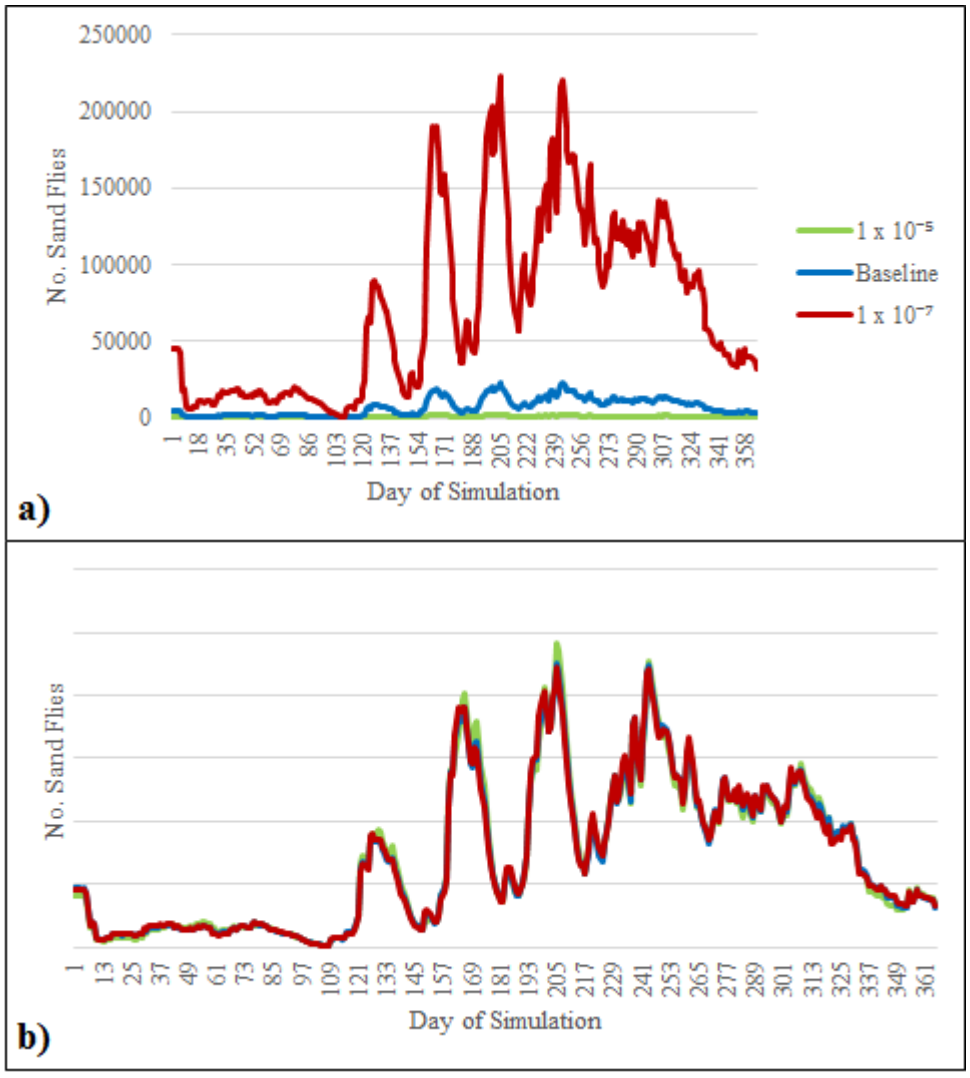


Figure 11: Comparison of **a)** simulated adult sand fly abundances and **b)** simulated adult sand fly seasonality, generated by three different values of the parameter controlling density-dependent mortality of larvae due to cannibalism ($r_{i,Can}$).

Validation

I validated the baseline model by (1) evaluating the ecological interpretability of seasonal trends in the simulated sand fly life cycle and (2) comparing simulated fluctuations in relative abundance of adult sand flies to fluctuations in relative abundance of adults caught in light traps in each of three villages in Bihar over a 12-month period. Simulated seasonal trends were representative of the general temperature-dependent trends characteristic of the sand fly life cycle in Bihar (Fig. 12). Simulated oviposition did not occur until mid-February (day-of-year 42), when temperatures first exceeded a suggested egg-laying threshold (15℃) (Kasap and Alten 2006), with the first mass emergence of adults occurring 85 days later during May (day-of-year 127), and the largest peak in adult abundance occurring during the latter portion of July (day-of-year 205), as observed by prior field researchers (Poche et al. 2011).

Simulated fluctuations in relative abundance of adults reflected the general variability in relative abundance of adults caught weekly in the three villages in Bihar, although, not surprisingly, trends in the field samples were less distinct (Poche et al. 2011) (Fig. 13a). Significant statistical differences in simulated and observed daily fluctuations in adult sand fly density were not found when performing a non-parametric sign test with a statistical probability of 0.95 (p= 0.1236; 0.5000; 0.0704). Although one village (Mohammadpur) showed significant differences with a statistical probability of 0.90 most likely due to reduced sand fly numbers during the months of September, October and November not observed in the other villages or the baseline simulation. The simulated mean monthly and mean quarterly abundances were generally similar to

observed monthly and quarterly means (Fig. 13b, c), although simulated adults appeared two months earlier (January and February) and did not exhibit the relatively large March increase observed in the field data.

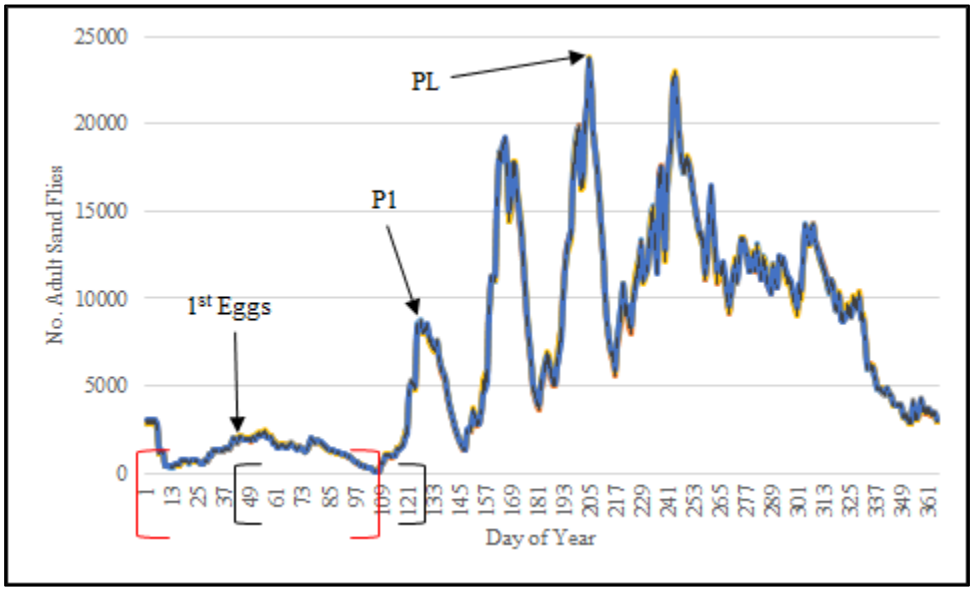


Figure 12: Simulated fluctuations in abundance of adult sand flies generated by the baseline model. Red brackets indicate a generation of overwintering sand flies. Black brackets indicate the time between initial oviposition and the first post-winter peak in abundance of adults (P1). PL indicates the largest peak abundance of adults.

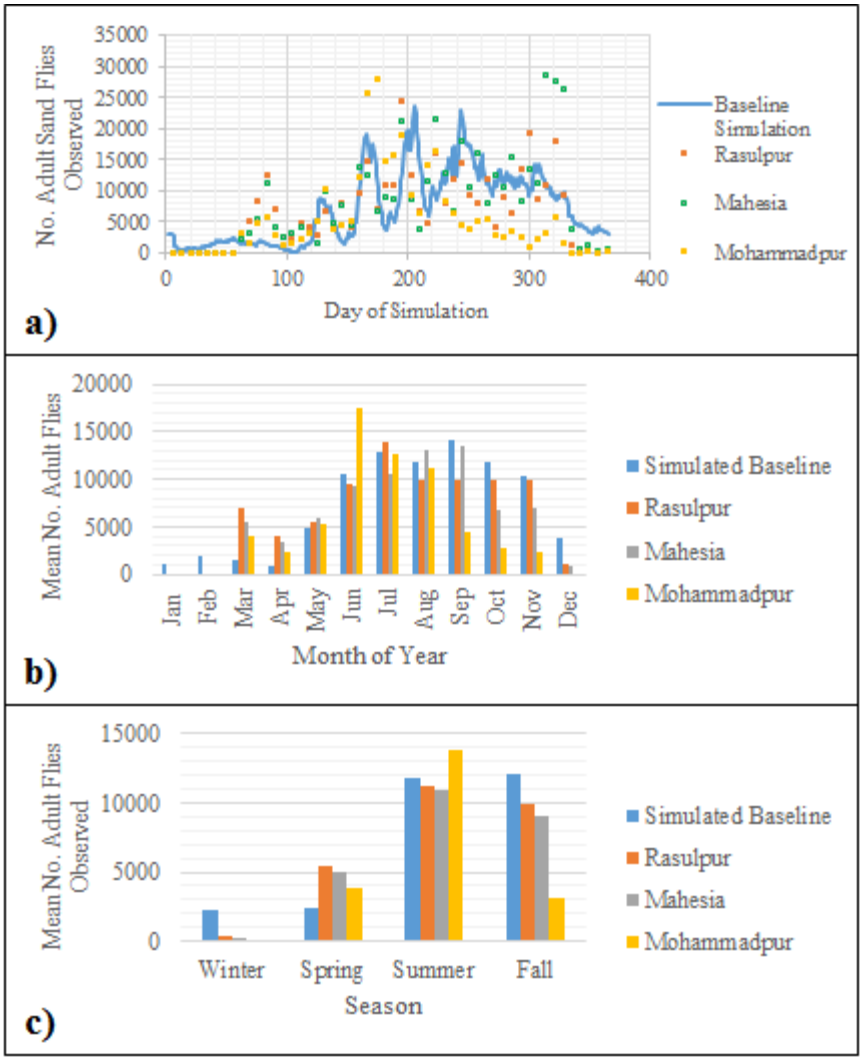


Figure 13: a) Comparison of simulated abundances of adult sand flies generated by the baseline model and relative numbers of adults caught in light-traps in three villages in Bihar, India (Poche et al. 2011). Also presented are comparisons of simulated versus observed **b)** mean monthly and **c)** mean quarterly abundances of adults. Village light traps catches (Rasulpur, Mahesia, Mohammadpur) were multiplied by several orders of magnitude (500, 280, 450) in order to make them comparable with the simulation.

Sensitivity Analysis

To assess the sensitivity of model behavior to my representation of the temperatures to which the various sand fly life stages were exposed, I ran a series of simulations in which I altered the baseline time series of organic matter or air temperatures. I first sequentially reduced the daily temperatures within organic matter, while maintaining the baseline seasonality, until the sand fly population could no longer survive. I then sequentially increased these temperatures until the population could no longer survive. I then repeated the same procedure with the daily air temperatures.

Simulated fluctuations departed noticeably from the baseline pattern and established a new dynamic equilibrium by the second year under each of the modified temperature regimes (Fig. 14). Populations were unable to survive when temperatures within organic matter were reduced or increased by >15%, or when air temperatures were reduced by >25% or increased by >10%.

When temperatures within organic matter were reduced by 15%, generation times were noticeably prolonged with the initial post-winter peak in adult abundance reduced and occurring much later due to increased development times and increased natural mortality of immature life stages (Fig. 14a). Only one generation of adults was observed when temperatures within organic matter were reduced by 15%.

When temperatures within organic matter were increased, post-winter peaks in adult abundance occurred earlier than at the baseline temperatures (Fig. 14b). However, adult abundances were reduced during summer when temperatures within organic matter were increased by 10% and 15% due to temperatures that approached the upper thermal

limit of larvae. Post-winter peaks in adult abundance were largest during the spring and fall when temperatures within organic matter were increased by 5% and 10% because these temperatures increased survivorship of immature stages.

When air temperatures were modified, timing of the post-winter peak in adult abundance was not affected noticeably, since the timing of this peak depends primarily on the development rates of immature life stages, which are driven by temperatures within organic matter, except when air temperatures were reduced by 25% (Fig. 14c). When air temperatures were reduced by 10%, adult survivorship during spring and summer increased, as did rates of successful oviposition, resulting in significant peaks in adult abundance during summer and fall, with the largest peak occurring in September. When air temperatures were reduced by 25%, adult abundance declined to nearly zero as a result of low rates of successful oviposition.

When air temperatures were increased, peaks in adult abundance were reduced because of increased natural mortality, but occurred in more rapid succession due to reduced pre-oviposition periods (Fig. 14d). Adult abundance was markedly reduced when air temperatures were increased by 10%, which approached the upper thermal limit of adults.

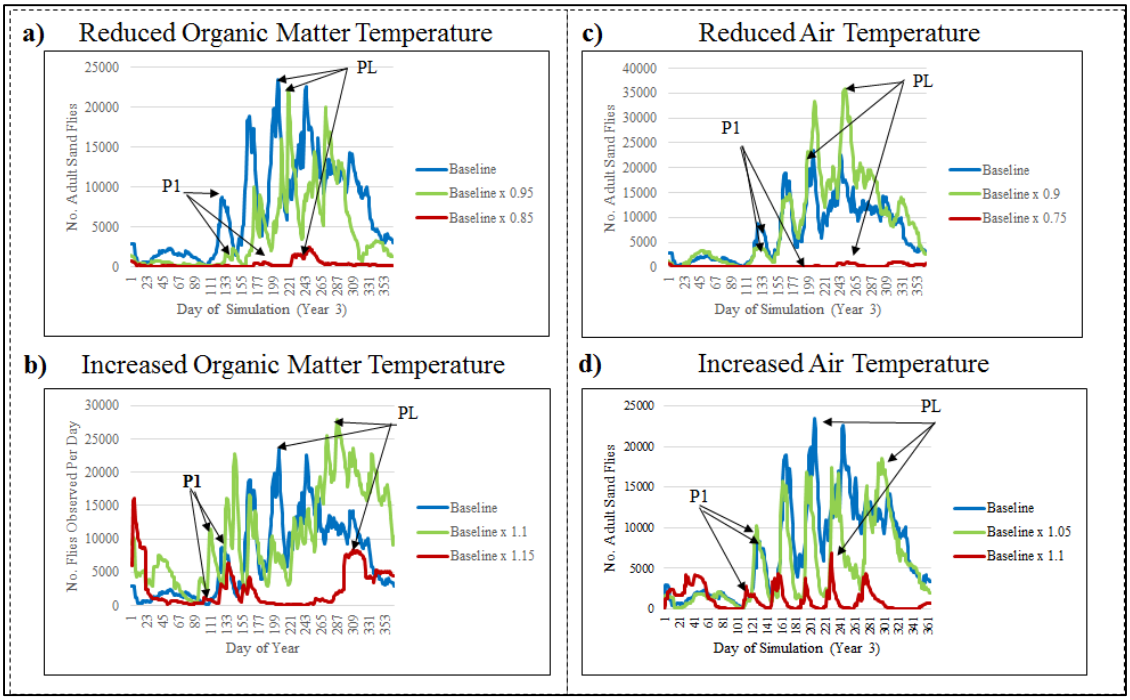


Figure 14: Comparison of simulated fluctuations in abundances of adult sand flies generated when the baseline model was modified such that temperatures within organic matter are **a)** decreased by 5 or 10%, or **b)** increased by 10 or 15%, or air temperatures are **c)** decreased by 10 or 20%, or **d)** increased by 5 or 10%. P1 indicates the first post-winter peak in abundance of adults and PL indicates the largest peak abundance of adults.

2.4 Simulated Response of Sand Fly Populations to Control Schemes Using Fipronil-Treated Cattle

Experimental Design

I assessed the potential efficacy of various treatment schemes using fipronil-treated cattle to control sand fly populations by running 16 sets of simulations in which I varied (1) the percentage of cattle treated and (2) the frequency of treatment application (Table 1). During each simulation, the baseline time series of organic matter and air temperatures were used, and the same percentage of cattle was treated at the same frequency for 3 consecutive years, during which time the abundance of adult sand flies was monitored. I assessed efficacy of the different schemes based on their ability to reduce the number of adult sand flies present during the third year of treatment application by >67% relative to the baseline (no control) simulation. Prior researchers estimated, using an SIR model, that >67% reduction in the adult sand fly population in Bihar would eliminate the threat of a VL epidemic (Stauch et al. 2014). For each simulation, I calculated both the mean number of adult sand flies present during the third year of treatment application and the mean number of days in which the adult population was not reduced by >67% relative to the baseline (no control) mean during the third year of treatment application.

Table 1: Experimental design consisting of 16 combinations of (1) percentage of cattle treated and (2) frequency of treatment application used to assess the potential efficacy of using fipronil-treated cattle to control sand fly populations.

No. Treatments Per Year	Cattle Treated (%)	Treatment Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
12	100	X	X	X	X	X	X	X	X	X	X	X	X
	75	X	X	X	X	X	X	X	X	X	X	X	X
	50	X	X	X	X	X	X	X	X	X	X	X	X
	25	X	X	X	X	X	X	X	X	X	X	X	X
6	100		X		X		X		X		X		X
	75		X		X		X		X		X		X
	50		X		X		X		X		X		X
	25		X		X		X		X		X		X
3	100					X		X		X			
	75					X		X		X			
	50					X		X		X			
	25					X		X		X			
1	100							X					
	75							X					
	50							X					
	25							X					

The X's indicate months in which treatments were applied.

Simulation Results

Five of the 16 simulated schemes reduced the mean number of adult sand flies present during the third year of treatment by >67% (Fig. 15a). To achieve >67% reduction, at least three treatments per year were required, with the required percentage of cattle treated decreasing from 100 with three or six treatments per year to 50 with 12 treatments per year. An additional three treatments achieved >50% reduction (treating 75% of the cattle three or six times per year, or treating 25% of the cattle 12 times per year). Eradication was achieved only by treating $\geq$75% of the cattle 12 times per year. Treatment applied once per year did not reduce the sand fly population by >50% regardless of percentage of cattle treated.

Treatments that resulted in >50% or >67% mean sand fly population reduction by the third year of treatment showed potential to reduce sand fly populations temporally (Fig. 15b, 16). During the third year of treatment, treatment applied 6 times per year to 100% and 75% cattle, and treatment applied 3 times per year to 100% and 75% cattle kept the sand fly population below the estimated epidemic threshold line (67% mean reduction) for all but 10, 72, 50, and 89 days of the third year, respectively. Treatment applied 12 times per year to 25% cattle reduced the sand fly population to below the epidemic threshold for all but 74 days of the third year and if 50% cattle were treated 12 times per year the population never exceeded the epidemic threshold after the first year of treatment.

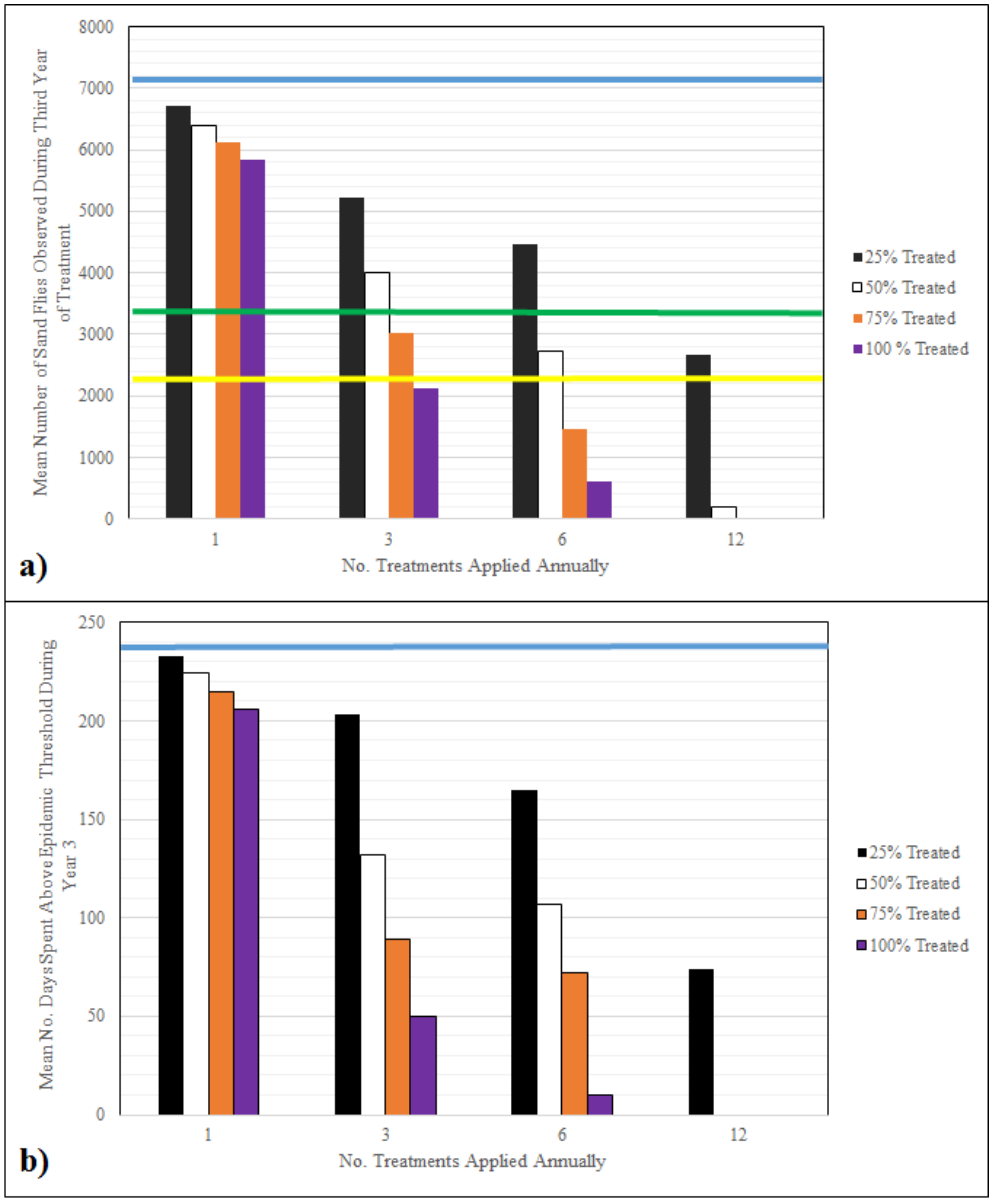


Figure 15: Comparison of **a)** mean abundance of adult sand flies during the third year of simulations and **b)** mean number of days that sand flies were not reduced >67% during the third year of simulations, representing each of the 16 combinations of (1) percentage of cattle treated and (2) frequency of treatment application. The blue line indicates mean abundance of adults during the baseline (no treatment) simulations in part **a,** and the mean number of days that sand flies were not reduced >67% during the baseline (no treatment) in part **b**, and the green and yellow lines in part **a** indicate abundances equal to 50% and 33% (67% reduction), respectively, of mean baseline abundance.

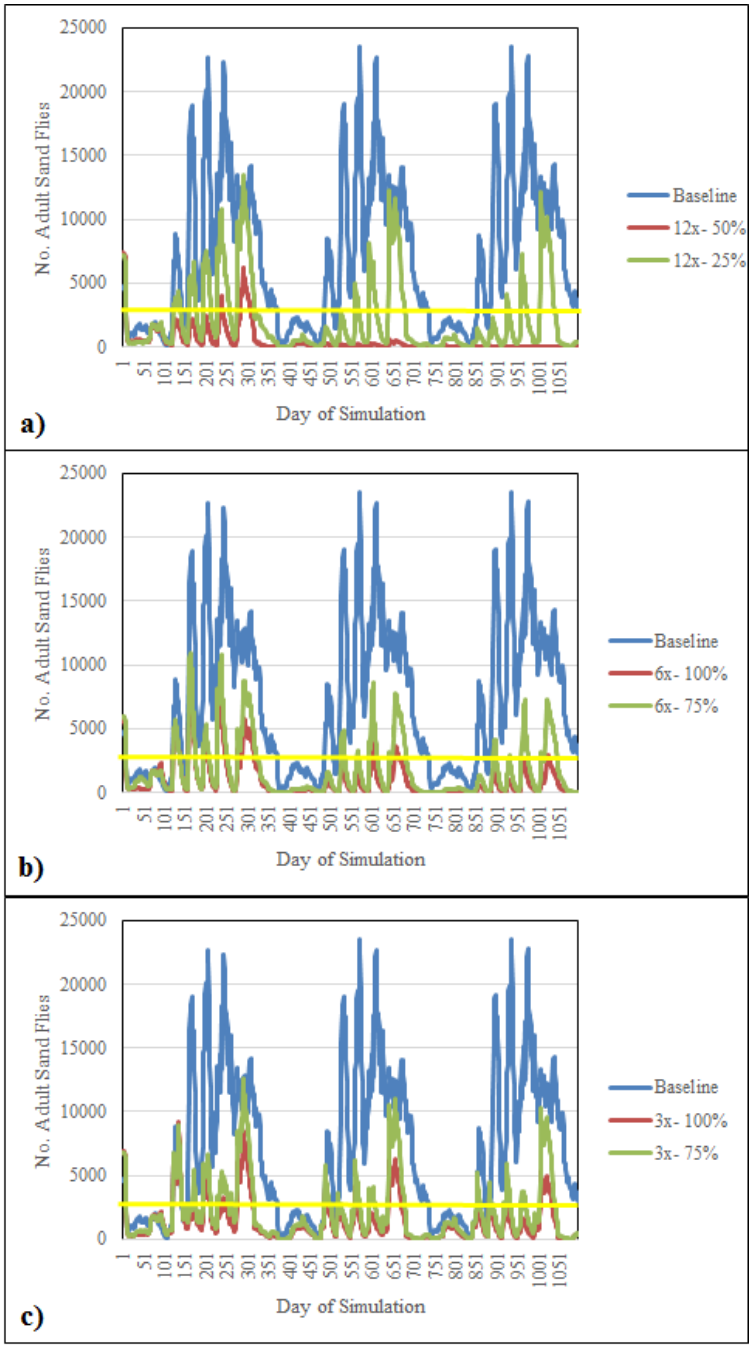


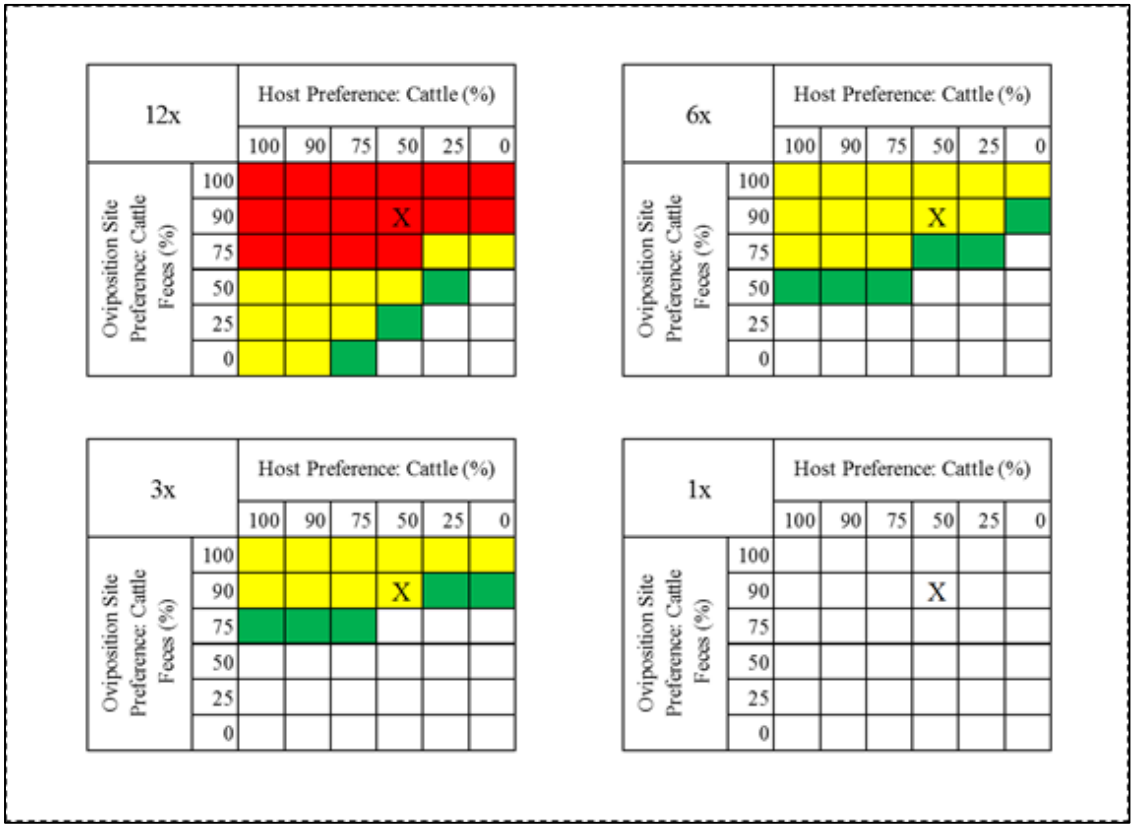
Figure 16: Comparison of simulated fluctuations in abundance of adult sand flies over a 3-year period in which **a)** 50 or 25% of cattle were treated 12 times per year, **b)** 100 or 75% of cattle were treated 6 times per year, and **c)** 100 or 75% of cattle were treated 3 times per year. The yellow line indicates an abundance equal to 33% (67% reduction) of mean baseline abundance.

Uncertainty Analysis

Two key assumptions under which I simulated the potential efficacy of using fipronil-treated cattle to control sand flies were (1) that 50% of adult flies obtained their blood meal from cattle and (2) that 90% of eggs were laid in organic matter containing cattle feces (Singh et al. 2008, Garlapati et al. 2012). To evaluate how uncertainty associated with these estimates might affect simulated treatment efficacy, I ran a series of simulations, at application rates of 1, 3, 6, and 12 times per year, representing all 36 combinations of 0, 25, 50, 75, and 100% of adults blood feeding on cattle and 0, 25, 50, 75, and 100% ovipositing in organic matter containing cattle feces. In all of these simulations, 100% of the cattle were treated over a three year treatment period.

Fipronil treatment, orally applied to cattle, was estimated to be far more efficacious when the percentage of sand flies ovipositing in cattle feces was high (Table 2). With an application rate of 12 times per year, sand fly populations were eliminated if 100% and 90% of sand flies oviposited in cattle feces, regardless of the host preference. Sand flies were eliminated with 75% of oviposition occurring in feces if $\geq$50% of blood feeding occurred on cattle. If 100% of the sand flies blood fed on cattle and no oviposition occurred in feces, the population was reduced by >67% but elimination did not occur. With application occurring 6 times per year, adult sand fly populations were reduced by >67 if 100%, 90% and 75% oviposited in cattle feces and a minimum of 0%, 25% and 75% blood fed on cattle, respectively. Sand fly populations were reduced by >50% when 50% oviposited in cattle feces and $\geq$75% blood fed on cattle. Sand fly populations were not reduced by >50% when <50% oviposited in cattle feces regardless of host preference.

Table 2: Comparison of mean abundance of adult sand flies during the third year of simulations in which 100% of cattle were treated 12, 6, 3, and 1 time per year, assuming the indicated percentages of adults obtained their blood meal from cattle and oviposited in organic matter containing cattle feces.



X indicates the parameter estimates for the previous experimental design involving 16 simulated treatment schemes.

When treatment was applied 3 times per year, the sand fly population was reduced by >67% if 100% and 90% oviposited in cattle feces and at least 0% and 50% blood fed on cattle, respectively. The sand fly population was reduced by >50% when 75% oviposited in cattle feces and at least 75% blood fed on cattle. Sand fly populations were not reduced by >50% when <75% oviposited in cattle feces regardless of host preference. When treatment was applied once per year, the adult sand fly population was not eliminated, not reduced by >67%, and not reduced by >50%, regardless of host or oviposition site preference.

CHAPTER III

CONCLUSIONS

3.1 Model Limitations

The model is limited by gaps in knowledge regarding certain elements of sand fly ecology. Through sensitivity analysis I have reinforced the importance of obtaining greater knowledge of temperatures of exposure and host and breeding site preference. These are important factors that will influence sand fly population dynamics and future success of sand fly control. These model limitations may also be some of the model's strengths in that they pinpoint areas in sand fly ecology to which research should be allocated.

True Temperatures to Which Sand Flies Are Exposed

The results of sensitivity analysis suggest that sand fly population dynamics may be greatly influenced by the temperatures to which sand flies are exposed in the field. Laboratory-reared sand flies display temperature-driven development, but we lack knowledge with regards to the specific temperatures these flies are exposed to in the field. The sand fly population does not survive at the time series of maximum observed temperatures recorded in the field, which comes as no surprise considering flies feed nocturnally in the wild, being observed blood feeding at night and early in the morning (Dinesh et al. 2001, Lemma et al. 2014, Gebresilassie et al. 2015a).

Oviposition Site Preference

The results of uncertainty analysis suggest that sand fly population dynamics is more greatly affected when sand flies oviposit in cattle feces at high percentages than when they blood feed on cattle at high percentages. Our lack of knowledge regarding the oviposition sites of sand flies allows us to only make estimates regarding the percentage of sand flies ovipositing in cattle feces. Although *P. argentipes* larvae in India are predominantly recovered from cattle feces, they are recovered in very small numbers (Dhiman et al. 1983, Ghosh and Bhattacharya 1991, Kundu et al. 1995, Singh et al. 2008). Globally, sand flies have been collected from numerous areas including animal shelters, leaf litter, and tree holes (Feliciangeli 2004). If sand flies are found to oviposit primarily in organic material independent of cattle feces, than it is possible that another form of larval control should be used to supplement fipronil-treatment.

Impact of Precipitation

The above model predicts sand fly population dynamics as a function of temperatures of exposure. But the model does not include other environmental factors such as precipitation. Results of field studies in India have concluded that sand fly population numbers may be correlated with increased rainfall. Bihar experiences heavy monsoon rains during the summer months with sand flies being most abundant during summer and fall. Studies suggest that monsoon rains may improve breeding habitat of sand flies and increase population abundance (Modi et al. 1978, Pandya 1983, Kumar et al. 1988, Ghosh et al. 1999, Poche et al. 2011). Other studies have suggested that sand fly numbers may be negatively correlated with heavy rainfall (Picado et al. 2010). The role

of precipitation in sand fly ecology is not yet understood and therefore I chose not to quantify and include it in the model. However, future attempts should be made to do so as this could play an important role in influencing sand fly population dynamics.

Blood Feeding Behavior

If this model is ever expanded to include a human population, for the purpose of representing VL epidemiology, we are limited by our lack of knowledge regarding the mechanisms that influence VL transmission. Little is known about the blood feeding behavior of sand flies in the field. Sand flies typically feed once before ovipositing and dying in the laboratory, but multiple blood meals are required in order for VL vector-transmission to occur. *P. argentipes* in the laboratory exhibit gonotropic concordance (taking one blood meal for each oviposition cycle). However, they can be gonotropically discordant at low percentages, in which they acquire more than one blood meal during a single oviposition cycle (Ghosh and Bhattacharya 1992). A single *P argentipes* female can take multiple blood meals from humans and other animals as determined by cytochrome *b* amplification and gel immunodiffusion performed on wild-caught *P. argentipes* females (Mukhopadhyay and Chakravarty 1987, Ghosh et al. 1990, Basak et al. 1995, Palit et al. 2005, Garlapati et al. 2012). Additional data on the feeding behavior of *P. argentipes* would help in estimating more reliable VL-transmission rates.

3.2 Model Usefulness

The model fittingly captures the population dynamics of sand flies in Bihar at temperatures recorded in the field. Most interestingly, the simulation model captures a decrease in sand fly populations in April followed by a sharp increase in May observed in three Bihari villages used for model validation and may provide a hypothesis for why these fluctuations occur (Poche et al. 2011). Simulation results suggest the population reduction in April to be a decline related to the die off of overwintering sand flies. The influx in numbers occurring in May is predicted by simulations to be the first generation of adult sand flies emerging from eggs having been laid in late winter.

Simulated fipronil-treatment suggested that sand fly populations recover quickly and that a single annual treatment will not sufficiently reduce sand fly numbers, regardless of the percentage of cattle being treated. The simulations were able to predict proposed treatment schemes most likely to be efficacious against sand fly populations. Maximum percentage of treated cattle and maximum number of annual treatment applications resulted in the greatest efficacy. However, the model suggested that sand fly populations can be reduced to below the estimated VL epidemic threshold (>67%) if treatment is applied 3 times per year with 100% cattle being treated. Bihar is by far the poorest state in India averaging $100 million gross domestic product in comparison to the Indian average of $274 million (Thakur et al. 2000). People within VL-endemic zones in Bihar are among the most impoverished people in the state and the world (Boelaert et al. 2009). Considering the socio-economic status of the villagers at risk, and the possibility of this form of treatment being commercially available to them,

it is more feasible to suggest treatment be applied 3-6 times per year. It is additionally encouraging that simulations predict fipronil-treatment may suppress sand fly numbers below the estimated epidemic threshold over the three-year treatment period. The VL pathogens *L. donovani* and *L. chagasi* can persist within hosts for lengthy periods (sometimes up to 2-3 years), suggesting a need for sand fly population density to be suppressed over a long-term period (Badaro et al. 1986, Singh et al. 2002).

Uncertainty analysis of the proportion of adults feeding on cattle and larvae feeding on cattle feces suggests that this form of treatment may be applicable at variable percentages of oviposition site and blood meal preferences, and therefore could be applicable under different ecological conditions, under which sand flies survive, in different parts of the world. Results suggests that fipronil is highly efficacious against larvae, resulting in mean sand fly reduction of >67%, if the majority of sand flies oviposit in cattle feces, even if little to no sand flies blood feed on cattle. Simulations suggest that this form of treatment will be very effective, if 100% cattle are treated and application is applied 3, 6 or 12 times per year. Simulations suggest that if treatment is applied 3-6 times per year a percentage of oviposition must occur in cattle feces (75%-50%) in order for mean sand fly reduction of >50% to be obtained. Simulations further predict that one treatment per year will have little impact on sand fly populations, regardless of host and oviposition site preference, further suggesting the need repeated annual treatment applications to suppress sand fly populations. Results also suggest that sand fly populations that feed heavily on cattle and do not oviposit in cattle feces can be reduced by >67%, but only if treatment can be applied 12 times per year. The sand fly

species, *P. orientalis*, the primary vector for VL in East Africa, has been reported to be highly zoophilic in Ethiopia, feeding primarily on cattle and donkeys (Gebresilassie et al. 2015c, Gebresilassie et al. 2015b). Another previously mentioned study out of India also suggested that *P. argentipes* has the potential to be highly zoophilic (Palit et al. 2005).

3.3 Recommendations

Simulate Variable Temperature Series and Include VL Pathogen

The above model uses two time series of temperatures that repeat every year, assuming that yearly Bihari temperatures to be relatively similar. It would be beneficial to further evaluate the model by exposing the sand flies to air and soil temperatures which vary each year. A scenario could be designed in which sand flies were exposed to a particularly cold or warm year with the impact on sand fly survivorship and population dynamics being evaluated. Predictions could be made with regards to how climatic changes such as temperatures to which the sand flies are exposed would impact the ability of sand flies to persist in Bihar.

Additionally, the above model ignores disease epidemiology in human populations, merely using prior model estimates (Stauch et al. 2014) as a benchmark to assess vector control success. As previously mentioned, SIR models representing VL epidemiology ignore the immature sand fly stages, often having a constant rate of emergence of sand flies, and do not focus explicitly on the control process. Ideally, the next step would be for the above model to be include a VL pathogen and additionally

represent VL epidemiology in a human population in Bihar. This would allow for VL transmission to fluctuate in response to the simulated temperature-dependent sand fly seasonality produced by the above model. Additionally, the impact of fipronil control on vector reduction, as a function of increased adult and larval mortality, and the role of vector reduction on the basic reproduction number could be examined simultaneously. This could produce more reliable estimates of VL transmission rates as well as more credible predictions of vector control success.

Expanded *P. argentipes* Lifetable

Far more data examining the temperature-dependent relationships of *P. papatasi* are available than are for *P. argentipes*. Development times have been shown to be largely similar for both species, but subtle differences have been observed in the laboratory (Ghosh et al. 1992). The lifetables for *P. papatasi* are more extensive than those produced for *P. argentipes*, giving us a better understanding of how *P. papatasi* function at colder temperatures (Guzmán and Tesh 2000, Kasap and Alten 2005, 2006).

The extent to which *P. argentipes* development and survivorship are effected at various temperatures may be best understood by exposing them to a wider range of constant temperatures and using the data generated to develop an extensive lifetable. I recommend performing an experiment, using *P. argentipes*, under the same laboratory conditions reported (Kasap and Alten 2005, 2006). This would require incubators to be set at six constant temperatures of exposure (15, 18, 20, 25, 28, 32 ℃). Relative humidity would need to remain constant (60%) as it could also influence developmental processes. *P. argentipes* cohorts would be monitored as they progress through the egg,

larval, pupal, pre-reproductive, pre-oviposition, reproductive, and post-reproductive stages. Insect processes of interest to be noted would be: the stage-specific development times and mortality rates, the total development time from egg-adult, the daily probability of blood feeding for pre-reproductive adults, the pre-oviposition period length, the number of eggs laid per reproductive female, the proportion of females acquiring a second blood meal, and the sand fly generation time. If *P. argentipes* are found to complete development at 15℃ then the study could be replicated at temperatures <15℃. It would also be of great interest to determine the upper and lower thermal limits of *P. argentipes* eggs, larvae, pupae, and adults to better understand the robustness of *P. argentipes* to extreme temperatures.

Further Breeding Site Surveys

It is widely believed that greater knowledge of sand fly breeding sites will be essential in terms of directing control efforts in the future (Alexander and Maroli 2003, Feliciangeli 2004, Romero and Boelaert 2010, Warburg and Faiman 2011). Results of the uncertainty analysis suggest that fipronil treatment applied to cattle 3-6 times per year has the potential to reduce sand fly populations below the estimated VL epidemic threshold, but only if at least 50-75% oviposition occurs in soil containing cattle feces. Therefore, it is highly recommended that breeding site work continue to be conducted in Bihar and other areas where VL is an issue.

The majority of breeding site work in India has been conducted in or directly adjacent to human dwellings and cattle sheds (Dhiman et al. 1983, Ghosh and Bhattacharya 1991, Kundu et al. 1995, Singh et al. 2008). Although the majority of

breeding site studies have yielded very small numbers of larvae, there are a few exceptions outside of India. One example of larger positive sample collection occurred in Italy and involved the collection of several hundred larvae from topsoil inside an abandoned shed (Bettini et al. 1986, Bettini and Melis 1988, Bettini 1989). The other involved the collection of over 2,000 larvae from forest floors in Panama (Hanson 1961).

This suggests a need to broaden our search for sand fly breeding sites to include not only village dwellings, but also dense vegetation and other organic-rich microhabitats adjacent to the villages. This should involve, but not be limited to: the floors and walls of cattle sheds, the floors and walls of human dwellings, poultry houses, tree buttresses, tree holes, rodent burrows, and palm tree pulp. The latter is included because researchers have collected sand flies from the tops of palm trees over 18 m tall (Poché et al. 2012). Information regarding soil type, location, soil temperature, soil moisture content, and soil organic matter content should be carefully noted. Determining the specific breeding site preferences of *P. argentipes* and other sand fly species will aid in future control plans, particularly those focusing on larval control.

Fipronil Field Trial

Ideally, a field trial, where village cattle are treated orally with fipronil would best validate the usefulness this model. After a pre-treatment light trap sampling period, during which adult sand flies would be collected, cattle would be orally administered the fipronil drug. Fipronil would be orally applied to all village cattle at treatment application frequencies ranging from 3-6 applications per year. The sand fly population would continue to be monitored via light trapping over the course of a treatment and

post-treatment period. The number of symptomatic human VL cases would also be monitored over the course of the study. A significant reduction in the sand fly population and instances of clinical VL would indicate fipronil treatment as an efficacious form of sand fly control in Bihar. Results of the treatment regime could ideally validate the results of the model, but more likely give us potential insight into how to adjust the various model parameters.

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APPENDIX A

DATA ANALYSIS

Table A1: Presenting the data points used to develop Equation 1 representing the daily development of sand fly eggs as a function of organic matter temperature.

Development of Eggs						
Temp °C	Mean Development Time (Days)	Range (Days)	Reference(s)	Total Degree Days	Value Used	Daily Contribution Toward Development
25.5	14	(10-17)	Ghosh and Bhattacharya 1989 (Table 1)	280.5	11	0.091
28.5	8	(5-12)	Ghosh and Bhattacharya 1989 (Table 1)	228	8	0.125
30.5	6	(5-8)	Ghosh and Bhattacharya 1989 (Table 1)	183	6	0.167

The mean development time was converted into the total degree days which were then converted into the contribution toward development daily. A lower range value was used at 25.5 ℃ in order to shorten the development times recorded at temperatures <25.5 ℃. This was done because when using the mean value to develop the equation, simulated development times at temperature <25.5 ℃ were unrealistically long when compared with development times recorded in the laboratory (Ferro et al. 1998, Guzmán and Tesh 2000, Kasap and Alten 2005).

Table A2: Presenting the data points used to develop Equation 2 representing the daily development of sand fly larvae as a function of organic matter temperature.

Development of Larvae				
Temp °C	Mean Development Time (Days)	Reference(s)	Total Degree Days	Daily Contribution Toward Development
18	206.74	Kasap and Alten 2005 (Table 1)	3721.32	0.005
25.5	25	Ghosh and Bhattacharya 1989 (Table 1)	637.5	0.040
30.5	15	Ghosh and Bhattacharya 1989 (Table 1)	457.5	0.067

The mean development time was converted into the total degree days which were then converted into the contribution toward development daily. The mean development time of *P. papatasi* larvae at 20 ℃ was used as the low value to better represent the noticeable non-linear decrease in the development time of larvae at temperatures $\leq$ 20 ℃ (Kasap and Alten 2005) and not captured by *P. argentipes* data (Ghosh and Bhattacharya 1989).

Table A3: Presenting the data points used to develop Equation 3, representing the daily development of sand fly pupae as a function of organic matter temperature.

Development of Pupae						
Temp °C	Mean Development Time (Days)	Range (Days)	Reference(s)	Total Degree Days	Value Used	Daily Contribution Toward Development
25.5	15	(11-20)	Ghosh and Bhattacharya 1989 (Table 1)	306	12	0.083
28.5	9	(5-12)	Ghosh and Bhattacharya 1989 (Table 1)	228	8	0.125
30.5	7	(5-9)	Ghosh and Bhattacharya 1989 (Table 1)	183	6	0.167

The development time was converted total degree days which were then converted into the contribution toward development daily. Range values below the mean development times at 25.5, 28.5 and 30.5 ℃ were used to develop the equation in order to reduce the development time at temperatures
<25.5 ℃. This was done because when the mean values were used simulated development times at temperatures <25.5 ℃ were unrealistically long when compared to development times recorded in the laboratory (Ferro et al. 1998).

Table A4: Presenting data used to develop Equation 4, representing the daily probability of obtaining a blood meal for pre-reproductive adult sand flies as a function of days post-emergence.

Days Post-Emergence	Flies Feeding (%)	Reference(s)	Probability of Obtaining a Blood Meal	Cumulative Probability
1	0	Srinivason and Panicker 1993 (p. 537)	0.001	0.001
2	3.33	Srinivason and Panicker 1993 (p. 537)	0.03	0.031
3	57.33	Srinivason and Panicker 1993 (p. 537)	0.57	0.601
4	24.89	Srinivason and Panicker 1993 (p. 537)	0.25	0.851
5	8.68	Srinivason and Panicker 1993 (p. 537)	0.09	0.941
6	2.44	Srinivason and Panicker 1993 (p. 537)	0.02	0.961
None	3.33	Srinivason and Panicker 1993 (p. 537)	0.039	1

The percentage of flies blood feeding per day post-emergence were converted into a daily probability of obtaining a blood meal. The daily probability of obtaining a blood meal was then converted into a cumulative probability in which the probability of obtaining a blood meal is additive each day post-emergence that a blood meal is not obtained. Green represents the data points used to develop the equation. A non-zero value was needed (0.001) to generate the curve.

Table A5: Presenting the data points used to develop Equation 5, representing the daily development of pre-oviposition adult sand flies as a function of air temperature.

Temp °C	Mean Development Time (Days)	Reference(s)	Total Degree Days	Daily Contribution Toward Development
18	13	Kasap and Alten 2006 (Table 3)	234	0.077
25	6.33	Kasap and Alten 2006 (Table 3)	158.25	0.158
32	4	Kasap and Alten 2006 (Table 3)	128	0.250

The mean pre-ovipostion period lengths were converted into the total degree days which were then converted into the contribution toward development daily.

Table A6: Presenting the data points used to develop equations 6a and 6b, representing the number of eggs laid per reproductive adult sand fly as a function of air temperature.

Temp °C	Mean No. Eggs Per Female	Standard Deviation	Reference(s)	Estimated No. Female Eggs	Values Used
15	0	0	Kasap and Alten 2006 (Table 3)	0	0.1
18	2.8	0.9	Kasap and Alten 2006 (Table 3)	1.4	3.6
28	44.08	7.79	Kasap and Alten 2006 (Table 3)	22.04	22.0
30	NA	NA	NA	NA	3.6
32	3.6	1.55	Kasap and Alten 2006 (Table 3)	1.8	0.1

To develop part **a** the number of eggs per female were estimated for temperatures 15, 18 and 28℃. The mean number of eggs laid per female were divided by two at 28℃ to represent the number of female eggs laid. The number of eggs laid at 18℃ needed to be modified (3.6) and a non-zero value needed to be used at 15℃ (0.1) in order to develop the bisymmetrical sigmoid curve. To develop part **b** the same parameter values were used to estimate the number of eggs laid per female at 28, 30, and 32 ℃. The resulting value produced by an equation in the functional form of part **a** was then subtracted from 25.1464684, producing an inverted sigmoid curve representing a decrease in the number of eggs laid per female as function of temperatures increasing from 28℃.

Table A7: Presenting the data points used to develop Equation 7, representing the daily proportional mortality of eggs as a function of organic matter temperature.

Mortality of Eggs				
Temp °C	Mean Development Time (Days)	Mean Proportion Dead	Reference(s)	Daily Proportion Dying
25.5	13	0.12	Ghosh and Bhattacharya 1989 (Table 1)	0.010
28.5	8	0.07	Ghosh and Bhattacharya 1989 (Table 1)	0.009
30.5	6	0.08	Ghosh and Bhattacharya 1989 (Table 1)	0.014

The mean percentage of eggs dead during development were converted the mean proportion of eggs dead. The mean proportion of eggs dead was then converted into the daily proportion of eggs dead $S = 1 - (1 - L)^{1/d}$, where L is the mean proportion dead d the mean development time.

Table A8: Presenting the data used to develop Equations 8a and 8b, representing the daily proportional mortality of sand fly larvae as a function of current organic matter temperature.

Mortality of Larvae				
Temp °C	Mean Development Time (Days)	Mean Proportion Dead	Reference(s)	Daily Proportion Dying
-6	NA	1	Theodor 1936 (Table 2)	1
25.5	24	0.15	Ghosh and Bhattacharya 1989 (Table 1)	0.007
28.5	19	0.09	Ghosh and Bhattacharya 1989 (Table 1)	0.005
30.5	15	0.16	Ghosh and Bhattacharya 1989 (Table 1)	0.012
36.5	NA	1	Theodor 1936 (p. 657)	1

The mean percentage of larvae dead during development were converted into the mean proportion of larvae dead. The mean proportion of larvae dead was then converted into the daily proportion of larvae dead $S = 1 - (1 - L)^{1/d}$, where L is the mean proportion dead d the mean development time.

Table A9: Presenting the data used to develop Equation 9, representing the daily proportional mortality of pupae as a function of organic matter temperature.

Mortality of Pupae				
Temp °C	Mean Development Time (Days)	Mean Proportion Dead	Reference(s)	Daily Proportion Dying
25.5	15	0.03	Ghosh and Bhattacharya 1989 (Table 1)	0.002
28.5	9	0.02	Ghosh and Bhattacharya 1989 (Table 1)	0.002
30.5	7	0.02	Ghosh and Bhattacharya 1989 (Table 1)	0.003

The mean percentage of pupae dead during development were converted into the mean proportion of pupae dead. The mean proportion of pupae dead was then converted into the daily proportion of pupae dead $S = 1 - (1 - L)^{1/d}$, where L is the mean proportion dead d the mean development time.

Table A10: Presenting the data points used to develop Equations 10a and 10b, representing the daily probability of mortality of adult sand flies as a function of air temperature.

Mortality of Adults			
Temp °C	Mean Development Time (Days)	Reference(s)	Daily Probability of Mortality
-2	NA	Theodor 1936 (Table 2)	1
0	2	Theodor 1936 (Table 2)	0.5
3	4	Theodor 1936 (Table 2)	0.25
6	7	Theodor 1936 (Table 2)	0.143
25.5	14	Ghosh and Bhattacharya 1989 (Table 1)	0.071
28.5	9	Ghosh and Bhattacharya 1989 (Table 1)	0.111
30.5	6	Ghosh and Bhattacharya 1989 (Table 1)	0.167
39.5	NA	Theodor 1936 (Fig. 3)	1

The mean longevity of adults during development was converted into the daily probability of mortality $1/d$ where d is the mean longevity of adults.

Table A11: Presenting data used to estimate Equation 11, representing increased larval mortality in the form of cannibalism, in response to increased larval density.

# larvae	Mean No. Cannibalized	No. Died and Uneaten	No. Pupated	Reference(s)
25	4	3.3	18	Srinivason and Panicker 1992 (Table 2)
100	37.3	7.7	55	Srinivason and Panicker 1992 (Table 2)
200	108	13.7	78.3	Srinivason and Panicker 1992 (Table 2)
400	234.3	19	146.7	Srinivason and Panicker 1992 (Table 2)

A linear relationship is observed at densities ranging from 25-200 larvae. I assumed a linear relationship between cannibalism and density and used the slope of the line to estimate increased rate in larval cannibalism in response to increased larval density. The slope of the density-dependent line was then calibrated to insure no impact on population dynamics was not affected by changes in larval cannibalism.

Table A12: Data used to develop Equation 12, representing the decline in fipronil efficacy in cattle blood as a function of days post-application.

Fipronil-induced Mortality of Blood Feeding Sand Flies				
Day post-application	Fipronil-induced mortality within 3 days (%)	Reference(s)	Mortality Probability	Daily Probability
1	85	Poche et al. (unpublished)	0.85	0.46867
3	69	Poche et al. (unpublished)	0.69	0.32321
5	53	Poche et al. (unpublished)	0.53	0.2225
14	44	Poche et al. (unpublished)	0.44	0.17574
21	23	Poche et al. 2013 (Figure 1)	0.23	0.08343

The percentage adult sand flies dying due to fipronil over a 3-day period was converted into the cumulative probability of mortality. The cumulative probability of mortality was then converted into the daily probability of mortality $S = 1 - (1 - L)^{1/d}$, where L is the cumulative probability of mortality and d represents 3 days. An exponential curve was then fit to represent the approximate decline from 0.46867 to 0.08343 over 21 days.

Table A13: Data used to develop Equation 13, representing the decline in fipronil efficacy in cattle feces as a function of days post-defecation.

Length of One Half-life (Days)	Reference(s)	Number of Half-lives	Number of Days Post-Defecation	Fipronil Efficacy
128	EPA (1996) EU (2011)	0	1	0.567
		1	128	0.284
		2	256	0.142
		3	384	0.071
		4	512	0.035
		5	640	0.018
		6	768	0.009

Using the reported half-life of fipronil, I developed an exponential curve where fipronil efficacy halved approximately every 128 days. The value 0.567 is the initial efficacy of fipronil in feces on Day-1 of treatment.

Table A14: Data used to develop Equation 14, representing the decline in fipronil efficacy in cattle feces as a function of days post-application.

Day Post-Application	Mortality (%)	Reference(s)	Mortality (Proportion)	Mean Days Until Death	Daily Mortality (Proportion)
1	100	Poche et al. 2013 (Figure 2, Table 1)	1	5.5	0.567123872
3	100	Poche et al. 2013 (Figure 2, Table 1)	1	6	0.535841117
5	100	Poche et al. 2013 (Figure 2, Table 1)	1	9.5	0.384151789
14	100	Poche et al. 2013 (Figure 2, Table 1)	1	17	0.237301414
21	100	Poche et al. 2013 (Figure 2, Table 1)	1	15.5	0.257036049

The percentage adult sand flies dying due to fipronil over a 3-day period was converted into the cumulative probability of mortality. The cumulative probability of mortality was then converted into the daily probability of mortality $S = 1 - (1 - L)^{1/d}$, where L is the cumulative proportion of mortality and d represents the mean number of days until death. A value of 0.99 was used in place of 1.0 to calculate the above equation because if L=1 the equation results in 0.

Table A15: Data used to estimate the proportion of adult sand flies obtaining a blood meal from cattle (50%).

Host Prefence of *P. argentipes*		
Host	Number of Positive Sand Fly Blood Meals	Reference
Human	119	Garlapati et al. (Figure 3)
Cattle	60	Garlapati et al. (Figure 3)
Human/Cattle	80	Garlapati et al. (Figure 3)
Human/Goat	15	Garlapati et al. (Figure 3)
Goat	8	Garlapati et al. (Figure 3)
Chicken	1	Garlapati et al. (Figure 3)
Human/Chicken	1	Garlapati et al. (Figure 3)
Cattle/Goat	4	Garlapati et al. (Figure 3)
Total	288	Garlapati et al. (Figure 3)

In total, 50% of sand flies took either a full or partial blood meal from cattle.

Table A16: Time series of temperatures used to represent the air temperatures to which adult sand flies were exposed.

Minimum Air Temperature (°C) Recorded Per Day												
Day of Month	Month of Year											
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1	11	14	19	24	26	31	28	31	26	25	21	16
2	11	14	19	24	26	30	27	29	27	25	21	16
3	13	12	20	24	26	30	27	28	27	25	24	15
4	12	12	20	24	26	30	28	28	28	25	21	15
5	12	12	20	24	26	30	29	29	27	25	21	16
6	11	12	20	29	26	31	30	29	26	26	21	16
7	10	12	20	27	26	31	30	29	26	25	21	15
8	10	16	20	27	26	28	28	27	25	25	21	17
9	10	16	20	27	26	30	27	28	27	25	21	15
10	10	16	20	27	26	31	27	29	27	25	21	15
11	10	16	20	27	27	30	27	29	27	25	18	15
12	10	16	25	27	24	29	27	28	26	25	18	16
13	9	15	25	27	27	27	30	28	26	25	18	15
14	9	15	25	27	26	27	29	27	26	26	18	14
15	12	15	25	27	26	28	29	27	29	24	18	15
16	12	17	25	27	29	30	28	29	29	23	18	16
17	12	17	26	27	27	31	28	30	28	23	22	15
18	11	16	26	27	27	30	27	30	28	21	18	15
19	11	19	26	27	30	27	29	29	28	21	18	15
20	11	19	24	27	29	28	33	29	28	20	16	14
21	10	19	24	27	29	28	28	29	28	21	16	16
22	10	19	24	27	29	29	27	29	25	21	16	15
23	11	19	24	27	30	29	28	28	28	21	16	15
24	11	19	24	27	29	30	28	28	28	21	16	14
25	10	19	24	27	29	31	29	28	27	21	16	14
26	10	19	24	28	29	30	29	30	26	21	16	15
27	10	19	24	26	29	30	29	30	26	21	16	16
28	10	19	24	26	29	32	29	29	25	21	16	16
29	10	NA	24	26	32	26	30	28	24	21	16	15
30	10	NA	24	26	33	28	30	30	25	21	16	14
31	10	NA	24	NA	33	NA	31	28	NA	21	NA	13

The following temperatures are a time series of minimum daily air temperatures recorded over a calendar year (Poche et al. 2011, Poche et al., unpublished data). Minimum temperatures are used because adult sand flies are nocturnal.

Table A17: Time series of temperatures used to represent the organic matter temperatures to which immature sand flies were exposed.

Minimum Air Temperature (°C) Recorded Per Day												
Day of Month	Month of Year											
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1	20	20.58741	22.04315	24.43406	27.02547	29.19391	30.04019	29.26605	27.14174	24.55471	22.13666	20.56565
2	20.00107	20.62503	22.10941	24.51972	27.10819	29.24546	30.04147	29.21508	27.05924	24.46892	22.07004	20.52984
3	20.00335	20.66377	22.17654	24.60569	27.19035	29.29556	30.04093	29.16269	26.97621	24.38344	22.00431	20.49517
4	20.00682	20.70362	22.24454	24.69193	27.27192	29.34421	30.03859	29.1089	26.89267	24.2983	21.93948	20.46163
5	20.01151	20.7446	22.31339	24.77841	27.35288	29.39137	30.03443	29.05373	26.80865	24.21352	21.87555	20.42925
6	20.01739	20.78668	22.38307	24.86512	27.43318	29.43702	30.02847	28.9972	26.72419	24.12912	21.81254	20.39801
7	20.02448	20.82986	22.45357	24.95203	27.5128	29.48115	30.02071	28.93934	26.63931	24.04513	21.75047	20.36794
8	20.03277	20.87414	22.52488	25.03911	27.59171	29.52373	30.01115	28.88018	26.55404	23.96156	21.68934	20.33902
9	20.04226	20.9195	22.59699	25.12634	27.66987	29.56474	29.9998	28.81974	26.46842	23.87845	21.62917	20.31126
10	20.05295	20.96595	22.66987	25.21368	27.74727	29.60417	29.98666	28.75805	26.38248	23.79581	21.56996	20.28467
11	20.06484	21.01348	22.74351	25.30112	27.82385	29.64199	29.97173	28.69514	26.29625	23.71366	21.51173	20.25925
12	20.07792	21.06207	22.81789	25.38862	27.89961	29.67819	29.95503	28.63102	26.20975	23.63203	21.45448	20.235
13	20.0922	21.11172	22.893	25.47616	27.97449	29.71276	29.93656	28.56574	26.12302	23.55093	21.39823	20.21193
14	20.10767	21.16243	22.96883	25.56371	28.04848	29.74567	29.91633	28.49931	26.03608	23.47039	21.34299	20.19004
15	20.12434	21.21417	23.04535	25.65124	28.12154	29.77691	29.89435	28.43177	25.94897	23.39043	21.28876	20.16933
16	20.14219	21.26696	23.12255	25.73872	28.19365	29.80646	29.87063	28.36314	25.86172	23.31106	21.23556	20.14981
17	20.16123	21.32077	23.2004	25.82613	28.26477	29.83432	29.84518	28.29346	25.77434	23.23231	21.18338	20.13147
18	20.18146	21.3756	23.2789	25.91344	28.33488	29.86047	29.81802	28.22275	25.68689	23.15419	21.13225	20.11432
19	20.20287	21.43144	23.35802	26.00061	28.40394	29.88489	29.78915	28.15104	25.59937	23.07672	21.08217	20.09836
20	20.22546	21.48828	23.43774	26.08762	28.47192	29.90758	29.7586	28.07836	25.51183	22.99992	21.03315	20.08359
21	20.24923	21.54612	23.51805	26.17444	28.53881	29.92853	29.72637	28.00475	25.42428	22.92381	20.98519	20.07002
22	20.27417	21.60493	23.59892	26.26103	28.60456	29.94771	29.69247	27.93022	25.33676	22.84841	20.9383	20.05765
23	20.30029	21.66471	23.68034	26.34738	28.66916	29.96514	29.65694	27.85482	25.24929	22.77372	20.89248	20.04647
24	20.32757	21.72545	23.76228	26.43345	28.73256	29.98079	29.61977	27.77857	25.16191	22.69978	20.84777	20.03648
25	20.35601	21.78714	23.84472	26.5192	28.79476	29.99466	29.581	27.7015	25.07463	22.62659	20.80414	20.02771
26	20.38562	21.84977	23.92764	26.60461	28.85571	30.00674	29.54063	27.62365	24.98749	22.55417	20.76161	20.02013
27	20.41638	21.91332	24.01103	26.68965	28.9154	30.01704	29.49869	27.54504	24.90051	22.48253	20.72018	20.01376
28	20.4483	21.97779	24.09485	26.77429	28.97379	30.02553	29.45519	27.4657	24.81372	22.4117	20.67987	20.00859
29	20.48137	NA	24.17908	26.85849	29.03086	30.03222	29.41015	27.38567	24.72713	22.34168	20.64067	20.00462
30	20.51558	NA	24.26371	26.94223	29.08659	30.03711	29.3636	27.30498	24.64079	22.27249	20.6026	20.00185
31	20.55093	NA	24.34871	NA	29.14094	NA	29.31556	27.22366	NA	22.20415	NA	20.00029

These temperature values were based on a time series of daily soil temperatures recorded in West Bengal, India over a calendar year (Ghosh et al. 1999). A cosine curve was developed to represent the temperatures.